THE GERMINATION REQUIREMENTS OF NATURALLY BURIED SEEDS WITH PARTICULAR REFERENCE TO FLUCTUATING TEMPERATURES.

BY

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A thesis submitted in partial fulfilment of the requirements of the degree of Doctor of Philosophy

in the Department of Biological Sciences,

Plymouth Polytechnic.

August 1984

Thanks to God for His help

and wisdom.

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ABSTRACT

An apparatus is described which enables a layer of soil, containing naturally buried seeds, to be subjected to controlled temperature fluctuations over the range 5 C to 30 C, in the presence or absence of light. Results obtained from the use of this apparatus are presented for fourteen species, all common constituents of the buried seed bank and nearly all weeeds of arable land or pasture. At least partial inhibition of germination by darkness is found in all fourteen species and stimulation of germination by temperature fluctuations in all but one. In several species strong inhibition by certain constant temperatures is overcome by the inclusion of these temperatures in a fluctuating temperature regime but in some cases germination is inhibited by very large fluctuations. In the field the temporal pattern of germination for six of the above species is recorded and related to variations in the environment. When soil moisture is maintained at field capacity the timing of germination can be predicted from the temperature response curves found in the laboratory. The initial flush in the field seems to be governed by chilling requirements.

There is some evidence that a requirement for temperature fluctuations may be induced by burial. Laboratory tests on harvested Rumex obtusifolius seeds suggest that the length of exposure to far-red light before burial affects the subsequent responsiveness to temperature fluctuations. The possible mechanisms underlying the germination responses of buried seeds and the ecological significance of the results are discussed.

ACKNOWLEDGEMENTS

I would like to thank Dr. K. Thompson for his invaluable advice and encouragement throughout this work. I am grateful to Dr. E. A. Vallis and Dr. G. D. Wigham for their advice on the design of the apparatus, and Mr. B. Selley and Mr. A. Hopkins for their technical assistance. The printing was carried out by Mr. S. Monk. Financial support was provided by Devon County Council.

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CHAPTER 1

INTRODUCTION

Scientists have a tendency to study isolated components of the ecosystem (e.g.seeds or fluctuating temperatures) and expect the results of their studies to relate to the system as a whole, having set aside many factors which are potentially of interest and importance to the study. The individual components of an ecosystem interact both in space and time within a coordinated set of laws. In the extensive work described by A.S.Watt(1947) on pattern and process in the plant community, he emphasised that the role of the scientist should be to try to understand how the shattered fragments of individual components are fused together into the original unity of the whole ecosystem. It seems that considerable effort, párticularly in the field of seed biology, has been put into studying the 'shattered fragments' without trying to relate them to the whole system.

Many of the conditions under which seed germination has been studied are so far removed from the seeds' natural environment that it could be argued that the results are of no value when interpreting germination responses in the field. Baskin and Baskin(1978(a)) suggest that the primary reason that many laboratory studies of seed germination have contributed very little to an understanding of germination ecology is that the experiments were not designed to account for the facts that: (1)environmental conditions in the habitat change constantly and that with regard to factors such as temperature there are annual seasonal cycles (2)seeds continuously undergo physiological changes in response to changing environmental conditions and thus their germination responses vary continuously and (3)most species have a definite germination season.

In some studies of germination ecology the above facts were taken into account by burying seeds in the field and recording the seedling

emergence patterns (Karssen 1981(a), Roberts and Lockett 1978 and Stoller and Wax 1974). However, artificial burial does not simulate the environmental conditions through which seeds pass between maturation on the mother plant and burial under the soil. Fenner (1980) for example, found that seeds placed under leaves acquired a light requirement. In the field many seeds are likely to lie under a leaf canopy before being buried.In order to get closer to reality it was hoped to be able to study naturally buried seeds in soil, in the laboratory, under a controlled environment similar to that found in the field.

The purpose of the work described in this thesis was (a) to compare the germination requirements of naturally buried seeds with those of harvested seeds,and (b)to investigate various factors which govern the time and place of buried seed germination in the field, with particular emphasis on the effects of fluctuating temperatures. Initially this involved designing an apparatus that could subject a layer of soil containing naturally buried seeds to controlled temperature fluctuations in the light and in the dark.If buried seeds show markedly different germination responses to those of harvested seeds then much greater caution must be taken when extrapolating the results of laboratory experiments to the field situation.

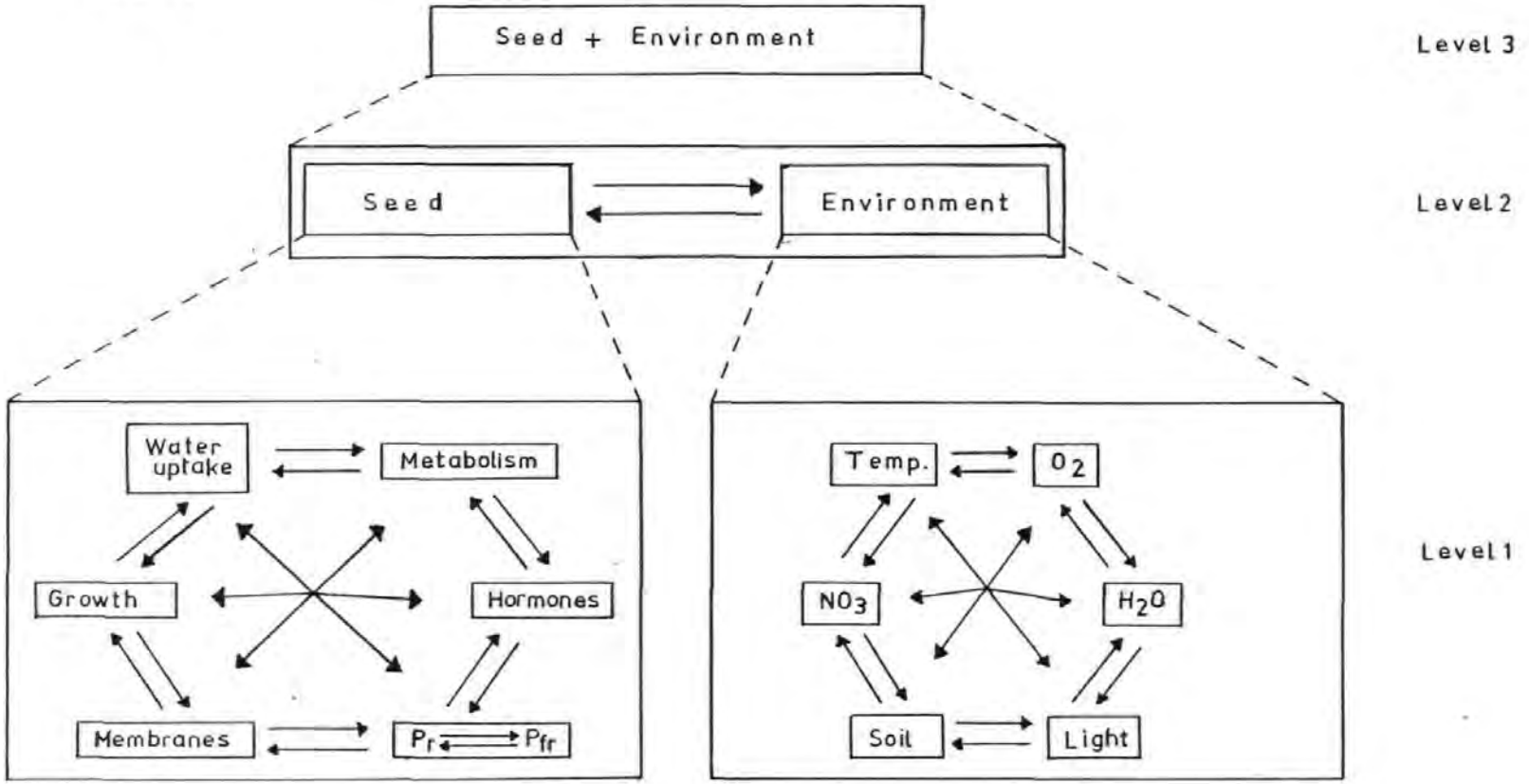
In order to start to design experiments that could meet the second aim described above, it was necessary to gain an understanding of seed biology and ecology. Much recent work on the germination responses of dormant seeds has emphasised the importance of interactions between different evironmental stimuli (e.g.temperature,light and nitrate) in promoting germination (Vincent and Roberts 1977, Roberts and Benjamin 1979). If evidence for seasonal cycles in the degree of response by buried seeds to these stimuli is

also taken into account (e.g.Karssen 1981(b)), then a very complex system starts to emerge. It would be useful to put all these interacting factors into some sort of framework, which is what modern systems analysis attempts to do (Simpson 1978).

System analysis states that all parts of a system are organised as a functional hierarchy of levels, which are in some way interrelated. The proper functioning on a given level requires that all levels below it function normally.Simpson (1978) stresses that the most satisfying description of dormancy is not necessarily the most detailed description at the lower biochemical level of the hierarchical structure but rather is the holistic view of the entire system, which brings an understanding of the relationships among levels as well as within levels.

The system under study in the present investigation can be described in the simplest way as ''Seed + Environment'' (Fig. 1.1). The system may be subdivided into two components, ''Seed'' and ''Environment'', with both contributing their own restrictions and rules to the system. Subdivision of both components at a lower level of the structure reveals separate environmental factors and biochemical processes in the seed. The system analysis allows one to be cautious against a reductionist approach, which would tend to concentrate on single factors and processes.

FIG. 1.1


Seed + Environment
Level 3
Seed
Environment
Level 2
Water uptake
Metabolism
Growth
Hormones
Membranes
Pr
Pfr
Temp.
O2
NO3
H2O
Soil
Light
Level 1

The remainder of this introduction aims to provide background information on the dynamics of seedling emergence from buried seeds upon which the hypotheses for the experimental work were based.

Seed banks

The presence of appreciable reserves of viable seeds in the soil is a feature of a wide range of plant communities. The term ''seed bank'' has been widely adopted to denote these reserves, which tend to decrease in size with increasing altitude (and, in northern temperate regions, with latitude) and in the later stages of plant successions (Thompson 1978). Weedy agricultural sites often contain vast numbers of buried seeds (up to 80,000m^2 in the plough depth) which remain viable for long periods.

These persistent seed banks confer the potential for regeneration in circumstances where disturbance of the vegetation is temporally and/or spatially unpredictable. Species which form transient seed banks in which no viable seeds remain for longer than a year, are adapted to exploit the gaps created by seasonally predictable damage and mortality in the vegetation.The functional significance of the different types of seed bank are discussed by Grime(1980) and Thompson and Grime(1979).Our main interest is in the pattern of emergence of seedlings from seeds of persistent seed banks.

Seed dormancy

The dormancy status of the seeds plays a large part in determining seed survival in the soil and patterns of seedling emergence. Primary or innate dormancy prevents germination during development and maturation on the mother plant and usually for some time after shedding or harvesting the seeds. Secondary or induced

dormancy develops after dispersal in seeds that are primarily non-dormant or have emerged partly or fully from primary dormancy. Primary induction while on the mother plant, as well as secondary induction in the independent seed may result either in full dormancy or in some form of relative dormancy. In a full or true dormant state, viable seeds are not able to germinate under any environmental conditions. Relative dormancy refers to states in which germination is limited to a certain range of environmental conditions. Alleviation of dormancy involves a widening of this range while induction of both primary and secondary dormancy involves a narrowing (Vegis, 1964).

The dormancy of seeds is subject to constant changes. If germination is confined to a certain range of conditions due to physiological limitations within the seed, a period of unfavourable conditions may narrow the range even further so that the relative dormancy increases. Roberts (1972) states that the persistence of dormancy after the return to conditions that were originally favourable for germination distinguishes secondary dormancy from enforced or imposed dormancy. Therefore, the latter situation is better referred to as an environmental inhibition of germination, which may enforce or impose secondary dormancy upon the seeds.

Seasonal patterns in seed dormancy

Fluctuations in the field behavior of seeds are governed by changes in the two components of the system: ''Environment'' and ''Seed''(Fig. 1.1). There are often marked seasonal fluctuations in the physical and chemical environment surrounding the seed. Periodicity in the microclimate at the soil surface and in the soil is governed either directly by changing environmental factors,such as

temperature and precipitation or indirectly by seasonal fluctuations in biotic and abiotic activities.

Patterns of change during burial of seeds in soil have been reported in several recent studies (Roberts and Lockett 1978, Baskin and Baskin 1978(b), Karssen 1981(a) and Roberts and Neilson 1982) in which buried seeds passed through a seasonal cycle of relief of dormancy, inhibition of germination and induction of secondary dormancy. This was characterised by periods of high light sensitivity and light insensitivity. In burial experiments with a span of more than one year the patterns of change in dormancy were found to repeat themselves in a similar way (Taylorson 1972, Karssen 1981(a)).

Seasonal fluctuations in dormancy were observed in both summer and winter annuals. Seeds of summer annuals are dormant in autumn, lose dormancy in winter and have it re-induced in summer, whereas winter annuals pass through these stages in spring, summer and autumn respectively. In general dormancy is released during the season preceeding the period with favourable conditions for seedling development, and is induced in the season preceding the period with harmful conditions for plant survival. Therefore the patterns of dormancy are of high survival value for the species. Germination only occurs in the field if the range of internal requirements of the seed overlap with the range of actual conditions in the habitat. The factors which govern the seasonal changes in dormancy have recently been reviewed by Karssen (1982) and are discussed in the following section.

Factors affecting dormancy state

Seeds of many species of summer annuals from temperate regions require a cold stratification or chilling treatment to break

dormancy.The length of the required cold period varies from a few days to several months. However, in a small minority of weed species it has been shown that a combination of low temperatures with light and/or nitrate is either indispensible or at least highly stimulatory to the loss of dormancy (Vincent and Roberts 1979, Roberts and Benjamin 1979).

In the seeds of winter annuals dormancy is broken during storage at high temperatures of 20°c, 25°c,30/15°c and 35/20°c (Baskin and Baskin 1976), and lower temperatures are ineffective or inhibitory. Experiments in which seeds were buried in the field and their emergence recorded in relation to environmental changes, confirm that the overlap between required and actual temperatures is the dominant factor in the determination of the suitable period for germination (Roberts and Neilson 1982, Baskin and Baskin 1980, Roberts and Lockett 1978). The initiation of germination in spring or autumn seems to depend on the passing of a certain minimum or maximum threshold temperature, respectively. Seedling emergence of several annual weeds in spring has, indeed, been related to soil warming (Stoller and Wax 1973). In some habitats lack of water restricts the period when conditions are suitable for germination (Baskin and Baskin 1979 and Newman 1963).

Other factors affecting seedling emergence

Although the majority of species show a broad seasonal pattern in the emergence of seedlings from buried seeds, some weedy species show small flushes of emergence throughout the year. This has been observed in natural seed populations (Roberts and Potter 1980, Benjamin 1974), in artificially introduced populations derived from bulk seed collections (Roberts 1964, Popay and Roberts 1970), and in

an artificially introduced population from a single plant of Capsella bursa-pastoris (Salisbury 1964). This intermittent germination is important in the survival of weed species but the causes are not very clear. Little is known about the relationship between conditions in the soil and the germination of seeds buried there.

Karssen (1982) suggests that the secondary dormancy of seeds near the soil surface may be removed by a thermal shock or a short exposure to anaerobic conditions (Le Deunf 1974) or dehydration (Karssen 1981(b), Kivilaan 1975). Such stress conditions occur particularly in the upper layer of the soil in dry-wet cycles which are part of many climatic regimes.

Seeds with different germination requirements may be found in different proportions in samples of seed from the same species at a particular site and this variability between seeds is particularly marked in weedy species. The range of germination requirements within a seed population must be an important factor concerned with the distribution of seedling emergence both in time and space.

As Harper, Williams and Sagar (1965) stressed, the soil is a highly heterogeneous and ever changing habitat to a weed seed. At this level little is known about the diversity of microenvironments within ordinary agricultural soil.This heterogeneity is likely to be such that if the climate changes almost imperceptibly, the environment of a number of seeds may be altered enough to encourage germination of a few of them. Changes of this nature may occur frequently so that, as observed, small flushes of germination occur throughout the year.

Seeds contain entire plants in an embryonic state. In order for them to ensure a high probability of survival for the plant which they produce, they should be capable of recognising the optimal conditions

for the establishment of the embryonic plant, first as a seedling and later in its more mature stages. Therefore, at some time before the seed has committed itself to the irreversible terminal processes of germination in a certain site, it must be capable of discriminating the potential of that site to provide the necessities which would increase the probability for successful completion of the life cycle.

Environmental requirements and seed perception

The autotrophic plant depends for its existence on several main components of the environment. These are water, energy, carbon dioxide, certain soil physical conditions, a balanced supply of minerals, and freedom from excessive competition.The perception and characterisation of the environment by the seed appears to depend on relatively few components, mainly temperature, light and water. However, by restricting perception to specific levels and regimes of these components, and to specific combinations or interactions of these few factors, a high degree of characterisation of the environment may be achieved.

It has already been noted that absolute temperatures play a large part in the seasonal emergence patterns of seedlings. Diurnal temperature fluctuations are also known to stimulate the breaking of seed dormancy in many species (Warington 1936, Thompson 1977 and Thompson and Grime 1983).

It seems likely that the response mechanism of germination to specific regimes of diurnally alternating temperature would be of value in characterising the microenvironment.

The amplitude of temperature fluctuations that a particular seed receives is governed partly by its depth of burial beneath the soil surface. The amplitude of fluctuations in soil surface temperature is

progressively damped with increasing depth due to the large heat capacity of the soil. It would be disadvantageous for the seed to germinate from a great depth because of limitations in the supply of stored food, which must sustain the seedling until it reaches the open air and becomes autotrophically established. There is evidence that harvested seeds of different species respond differently to different amplitudes of fluctuating temperatures (Totterdell and Roberts 1980, Thompson, Grime and Mason 1977 and Thompson and Grime 1983) but this has not been studied in buried seeds.

Sensitivity to temperature fluctuations in darkness may also act as a mechanism allowing buried seeds to detect gaps in the canopy of foliage and litter, thus ensuring light and freedom from excessive competition for the seedling. Foliage and litter may exert a very great insulating effect on the soil and diurnal temperature fluctuations occuring in bare soil are much larger than those experienced by soil beneath a closed canopy (Thompson 1977, Thompson Grime and Mason 1977).Many weedy species such as Holcus lanatus, Stellaria media and Rumex obtusifolius seem to be adapted to exploit gaps in sown pastures (Thompson and Grime 1983).

Light

It is of obvious value for the survival of a species to have seeds which are equipped with some means which will enable them to sense and assess the light - environment, both quantitatively and qualitatively, and to control their germination accordingly. It has been discovered that light can affect germination by its intensity, spectral composition and duration. All these characters are quantitative, the more of them the seed can perceive and the greater the precision with which it can adjust its response to their level,

the more precise will be its capacity to discriminate features of its environment which may be essential for survival.

The observation that dormancy could be removed by orange-red light (ca. 600-700nm.) but re-imposed by far-red light (ca. 720-780nm.), led to the discovery of the phytochrome system. Phytochrome exists as two interconvertible forms, Pfr, which is transformed by far-red light and Pr, which is transformed by red light. Pfr is the biologically active form and is required for germination. The properties of this system have recently been reviewed by Smith and Kendrick (1976) and Bewley and Black (1982).

This system may act as a depth sensing mechanism for seeds, as soil affects the quality of the penetrating light, reducing the transmission of the shorter wave-lengths more than that of the longer wave-lengths.The ratio of red/far-red light is thereby altered (Frankland 1981).

It is known that the seeds of many species develop a light requirement during burial and that a flush of seedling emergence often follows soil disturbance (Wesson and Wareing 1969(b)). The phytochrome system may also affect this response. If the light to which seeds are exposed at the soil surface has passed through a leaf canopy it is relatively rich in far-red wave-lengths and has a low red/far-red ratio (Holmes and McCartney 1975). A survey by Gorski, Gorska and Nowicki (1977) showed that of 30 species of light-requiring seed, the germination of 29 was greatly reduced by canopy light, and 9 out of 15 negatively photoblastic species were inhibited.

Seeds on the soil surface will remain inhibited until such time as a light environment becomes available which is relatively rich in red light, and which can therefore support photosynthesis and seedling establishment. Germination beneath established plants, which clearly

would be an unfavourable situation, can be prevented. Seeds of species which are able to colonise shaded sites are less sensitive to far-red rich canopy light (Silvertown 1980). Differences in sensitivity among seeds of different species is probably an important factor influencing species distribution.

A large proportion of the seed population in the soil is located at depths to which no light penetrates naturally. Koller (1972) suggests that the light requirement often only makes its presence felt under stress conditions such as supra-optimal temperatures and that the absence of light means that the limits of the environmental complexes in which seed germination will be possible will be considerably narrowed around the optimum. The seeds' awareness of conditions of stress, such as low soil-water potential, presence of toxic or inhibitory substances will be amplified.

Some buried seeds may have an obligate requirement for light, particularly small seeded species (Thompson and Grime 1983). It must also be remembered that photosensitivity in many species is temperature dependent and that the light requirement can often be bypassed by chilling or alternating temperatures. It can also be modified by interaction with other factors, e.g. nitrate or ethylene (Karssen 1981(b)).

Water relations

Water is essential for the rehydration of the seed as an initial step in its germination. The absolute amounts which are required are minute, not exceeding two or three times the dry weight of the seed. However, the subsequent growth of the seedling requires not only large amounts of water but also a sustained supply. As a result, control of germination by the water relations of the seed is of survival value

only if the mechanism provides additional information on the environment, other than that there is sufficient water for adequate rehydration of the seed itself.

Several mechanisms have been described which may act as a kind of rain-gauge which determines whether germination will occur at the proper time and place (Koller 1972, Mayer and Poljakoff-Mayber 1975). Many workers agree that, after the spring flush of germination, rainfall has an over riding influence on the timing of seedling emergence (Stoller and Wax 1973, Egley and Williams 1979, Roberts and Potter 1980). However, others have found little effect of soil moisture and point to diurnal temperature fluctuations as the main controlling factor (Yamamoto and Ohba 1977, Watanabe and Hirokawa 1975). It is very difficult to separate the effects of temperature and moisture in field experiments.

Soil physical conditions

Soil physical conditions are important for survival because they determine soil - plant - water relationships, soil aeration, and the mechanical impedance to root and shoot growth. It would therefore be beneficial for the plants to have seeds which are able to sense and evaluate the relevant physical properties of the soil and control germination accordingly.

There is evidence for this in that the germination of light-independent seeds, which are capable of germination in darkness, is also inhibited during burial (Holm 1972). Lonchamp and Gora (1979) suggested that this could be due to decreased oxygen tension. Although this may be important in water-logged soils or in the immediate vicinity of a seed, it has been found that the level of oxygen in a sandy loam soil rarely falls below 19% (Karssen 1981(b)).

Supply of minerals

A balanced supply of minerals is obviously essential to the successful growth of any plant, but there is little evidence for seeds being able to sense their mineral environment. One exception to this is the nitrate ion which has been reported to exert a marked stimulation of germination in a number of species (Vincent and Roberts 1977).

Interactions

It is clear from the preceeding sections that seeds can sense and assess many different factors in the environment. The more of these factors that are optimal for plant growth of that species, the greater the probability that a seedling produced by an individual seed will survive to be a mature plant. It is therefore not surprising that it has been found that one factor reinforces the effect of another in releasing seeds from dormancy. For example, seeds of some species do not respond to light at constant temperatures but do so at alternating temperatures (Thompson 1974, Vincent and Roberts 1977).

Interactions between temperature, light and nitrate have been investigated by Roberts and his colleagues in seeds of eleven weed species (e.g. Popay and Roberts 1970, Roberts and Benjamin 1979, Vincent and Roberts 1979).The many interactions shown by these species emphasise the fact that the release from dormancy is not necessarily the prerogative of a single factor, but that the combined action of several may be required. Indeed, in nature this may be the more common occurrence. This, of course, applies to the population as a whole and it is not yet clear how individual seeds react to single or multiple factors.

It is likely that the distribution of seedling emergence in the field, both in time and space, does not only depend on the heterogeneity of the microenvironments in which seeds are placed. It must also depend on the heterogeneity of the degree of dormancy within a population of seeds from a single species. The degree of dormancy shown by an individual seed is influenced by many interacting factors, both genetic and environmental, some of which are discussed below.

Effects of the maternal environment

When the variation in germination and dormancy of harvested seeds is examined it is found that the seeds of many species fall into two or more discontinuous populations —this is generally called polymorphism. Polymorphism is widespread in the Compositae, Cruciferae and Chenopodiaceae.

Cavers and Harper (1966) found differences in dormancy between seeds of different plants of Rumex crispus from the same site and also distinct differences between the seeds from individual panicles of a single plant. In some species the level of dormancy can be predicted from the physical appearance of the seed, e.g. Chenopodium album (Williams and Harper 1965), but little is known about its control. Clearly the regulation is both genetic and positional. It is interesting to note that the weedy members of a family (e.g. the Compositae) commonly have a higher incidence of polymorphism than the non-weedy members (Harper 1965).

Populations of seeds also show continuous variation in dormancy characteristics. This has been attributed to the variations in environmental conditions in which the mother plant grew. Grantlipp and Ballard (1963) found an increase in dormancy of harvested seeds was associated with low temperatures experienced in the maternal

environment. The length of photoperiod in which seeds develop and mature affects their subsequent dormancy (Gutterman 1973), as does the wavelength of light filtering through the seed coverings (Shropshire 1973, Cresswell and Grime 1981).

Effects of environment during seed dispersal and burial

Previous sections of this introduction have shown that seeds can sense several different factors in their environment. These factors affect biochemical systems in the seed which govern its dormancy state. It is likely that these systems (e.g. phytochrome equilibrium, membrane structure, hormone levels) are changing continuously in response to environmental variables even when the seed is in an apparently inert dormant state.

The length of time a particular seed spends under a leaf canopy before burial will affect its subsequent dormancy state by altering the phytochrome system (Gorski 1975).

The effectiveness of canopy light in inducing dormancy also depends on the density of cover. This has been simulated experimentally by Frankland and Poo (1980). The light-requiring dormant seeds of Plantago major,when spread on the soil surface,are stimulated to germinate when the overlying canopy is not dense, as expressed by leaf area index. As the latter increases, a decreasing proportion of the seeds are promoted untill finally there is barely any stimulation above the dark control. These responses reflect the decreasing red/far-red ratio which occurs as the leaf canopy thickens.

The canopy will also vary with time and space in many field situations ——in deciduous forests as the leaves appear and later drop, and under herbaceous plants as the leaf area index increases and then falls with senescence and death. Thus seeds on the soil surface

underneath such plants, experience a changing light environment and consequently a changing pattern of response in their germination.

Each seed, as it lies on the surface and is then buried will be in a slightly different microenvironment. The differences in temperature and moisture content of the seeds is known to affect the rate of induction of secondary dormancy (Totterdell and Roberts 1979), presumably by altering the rates of chemical reactions within the seed. It seems likely therefore, that the response of a particular buried seed to a change in its environment (e.g. a temperature shift), depends on the cumulative effects of all the previous stimuli to which it has responded since its initial development. This would depend on the ability of seeds to record and 'remember' in some way the effects of past stimuli. From a functional viewpoint, a seed 'memory' may reduce the probability of haphazard germination. For example, the longer seeds are subjected to far-red light before burial the less likely they are to respond to brief periods of white light if accidently unearthed (Gorski 1975). This would protect a large proportion of seeds from germinating if they were unearthed and quickly reburied (e.g. during ploughing).

However, if seeds 'remember' favourable stimuli such as light containing a high proportion of red wavelengths or a period of chilling for long periods, they may then germinate under unfavourable conditions. Baskin and Baskin (1972) found that seeds of Draba verna (a winter annual) that were buried immediately after shedding in Spring did not germinate in autumn unless they were unearthed in the light.If imbibed seeds received light anytime in late spring and summer and then became buried they germinated well in darkness when favourable temperatures were experienced in autumn. The light stimulus was retained for up to four months of burial under

unfavourable conditions for germination. This would seem to be disadvantageous as mature Draba verna plants are restricted to open, well lighted habitats such as rock outcrops.

In contrast to the long retention of a light stimulus, evidence has been found for several weed species that the chilling stimulus is only retained for a few days after the end of a cold period (Vincent and Roberts 1979).

Little work seems to have been done on the rate of deterioration of the effects of initial stimuli on seeds when they are subsequently buried under different conditions. It is known that dry stored seeds show a gradual reduction in dormany with increasing time of storage. This process, called after-ripening, widens the temperature range over which seeds can subsequently germinate and may cause an increased responsiveness to light and alternating temperatures (Cavers 1974, Roberts and Smith 1977). However in the natural environment of temperate climates, seeds are never dry for longer than a few weeks.

Organisation of thesis

Having reviewed the background information available, four main factors seem to be important in governing the time and place at which a seedling emerges from a buried seed in the field. These are: (a)heterogeneity in the seed microenvironment, (b)the non-uniform distribution of buried seeds, (c)intraspecific heterogeneity in dormancy states and (d)seasonal cycles in dormancy states, depending on the species.

In this investigation the effects of temperature fluctuations in the light and dark, on naturally buried seeds of various species, were studied. The study was approached from three directions.

(1) An apparatus was designed that could subject soil containing

naturally buried seeds to various temperature regimes in the light and dark. The soil preparation technique eliminated factor (b) and enabled factor (a) to be controlled to a large extent. When studying a particular species, factor (c) could be kept fairly constant by comparing soil samples from the same site. Factor (d) could be studied by collecting soil from a particular site at different times of the year and comparing seedling emergence patterns under the same temperature and light regimes.This is described in Chapter 4.

(2) Observations of seedling emergence were also made under field conditions and conditions where the above factors were controlled to some extent, while the soil received a natural field temperature regime which was recorded. The data from these experiments was interpreted in the light of knowledge gained fron the thermogradient bar apparatus.

(3) The mechanisms underlying some of the responses observed on the thermogradient bars and in the field were studied in more detail by subjecting harvested seeds of Rumex obtusifolius to controlled conditions in the laboratory. There are difficulties in relating petri-dish experiments to field conditions. Single factors which are found to influence seed germination in the laboratory are also likely to affect it in the field, but their relative importance will be modified by many other variables in the field.

Rather than dividing the thesis into three main parts to describe tests using the thermogradient bars, field work and then the other laboratory studies, an initial chapter on the design and applications of the thermogradient bars is followed by sections covering the various aspects of seed ecology which were investigated. Each section includes evidence which is drawn from both laboratory and field work. Thus all the work that provided evidence for the existence of seasonal

dormancy cycles in buried seeds is drawn together in Chapter 4.

This arrangement of the work allows a more ordered arguement to be built up (and hopefully makes the thesis more readable!). The aim is that the earlier chapters on the differences in germination responses between harvested and buried seeds and seasonal variations in the germination responses of buried seeds should be used as a necessary background to the main investigation described in Chapter 5. This chapter deals with the results of germination tests on the thermogradient bars for naturally buried seeds of 14 species, some of which were only tested on one particular sampling date.

The rather tentative ideas on the germination niche theory (Grubb 1977) contained in Chapter 6 and the petri dish experiments using harvested Rumex obtusifolius (Chapter 7) can be viewed in a correct perspective when related to the preceeding chapters.

In order for each chapter to stand largely on its own, some repitition of methods and data was necessary. For example, the responses of individual species to the temperature regime on the thermogradient bars are discussed in Chapter 5 and some of these data are used in a different way in Chapter 6 when comparing the germination responses of different species found together in the buried seed population at a particular site. The final chapter serves to relate the various observations on seed germination responses to the overall environmental control of buried seed germination.

CHAPTER 2

DESIGN AND USE OF THE THERMOGRADIENT BAR APPARATUS

Introduction

A thermogradient bar consists of a bar of heat conducting material which is heated at one end and cooled at the other, thus producing a gradient of temperature along the length of the material.The whole apparatus is thoroughly insulated against heat loss which could otherwise cause the gradient of temperature to be uneven or promote a gradient of temperature accross the width of the bar. They have been used in the determination of temperature optima and limits for insects (Coulianos 1955), algae (Halldal and French 1958), seed germination (Larsen 1965, Wagner 1967) and root growth (Barbour and Racine 1967). More recently they have been modified to provide a range of diurnal temperature fluctuations (Grime and Thompson 1976) and much standardised information is now available concerning the germination responses of many species to different amplitudes of temperature fluctuation (Thompson and Grime 1983).

The design of Grime and Thompson (1976) was modified as described in this thesis to enable a layer of soil, containing naturally buried seeds, to be subjected to controlled temperature fluctuations and moisture in the presence or absence of light. The seeds were to be maintained, as far as possible, in the same dormancy state in which they were found in the field situation. The light conditions were intended to simulate the field situation in which buried seeds had recently been exposed to light, e.g. by ploughing or the burrowing activities of moles. The dark conditions were intended to simulate the environment of seeds that had remained buried for many months and had not received light for this period.The sampling technique, therefore, had to enable those soil samples which were subsequently to be tested in darkness to be collected in the dark and the seeds to be protected from any light stimulus throughout the

course of the experiment.

As the main aim was to maintain buried seeds in their natural dormancy states and then to determine their germination requirements, no attempt was made to extract seeds from the soil. The techniques used to extract seeds from the soil usually include a floatation solution which might well alter the dormancy state of the seeds and also wet sieving which would have to be done in the light (Roberts 1981).

Thompson (1977) had attempted to use soil on his thermogradient bar but found difficulties with the soil drying out, very low numbers of seedlings emerging from the soil samples used and (in experiments in darkness) with the seedlings being very etiolated and therefore difficult to identify.These problems had to be overcome before reliable data could be collected concerning the germination requirements of buried seeds.

Design and construction of the thermogradient bar

Photographs of the thermogradient bar in use can be seen at the back of the thesis (Plates 1,2 and 3)

Size of the thermogradient bar

This was determined by a number of different factors.The size of soil sample placed on the bar had to be large enough to contain sufficient viable seeds to produce sufficiently large numbers of seedlings to allow valid statistical tests. The layer of soil had to be thin enough to be uniformly affected throughout its depth by the temperature fluctuations, and for the majority of it to receive light. Space for several replicates of each temperature treatment was also

required.

In Britain a survey of 58 vegetable fields gave a range of 1600-86 000 viable seeds m^{-2} in the top 15cm of soil, with a median value of 10 000m^{-2}(Roberts and Stokes 1966). Samples from 32 cereal fields in the English Midlands gave a range of 1800-67 000 viable seeds m^{-2} in the top 15cm, with a median of 5500m^{-2}(Roberts and Lockett 1976). The numbers of seeds found under grassland were very variable, ranging from 400m^{-2} in a permanent pasture to 70 000m^{-2} in a pasture that was formerly arable (Thompson 1978). Using the above figures as a rough guide it was calculated that a sample of soil a metre square and 0.5cm thick would contain sufficient seeds for six replicates of the temperature treatment, providing soil was taken from sites in the upper 50% range of seed densities. A large number of replicates were required because of the heterogeneity of the germination requirements found in a buried seed population.

Thus a large area of soil was to be spread onto a flat aluminium sheet which projected at each end through a water - tight seal into a water - jacket. The feasibility of producing a regular temperature gradient between 5°C and 25°C with such a system was discussed in detail with a physicist (* see footnote). The water pumping system was designed to prevent exessive loss of heat as the water travelled accross the width of the bar (Fig.2.1).

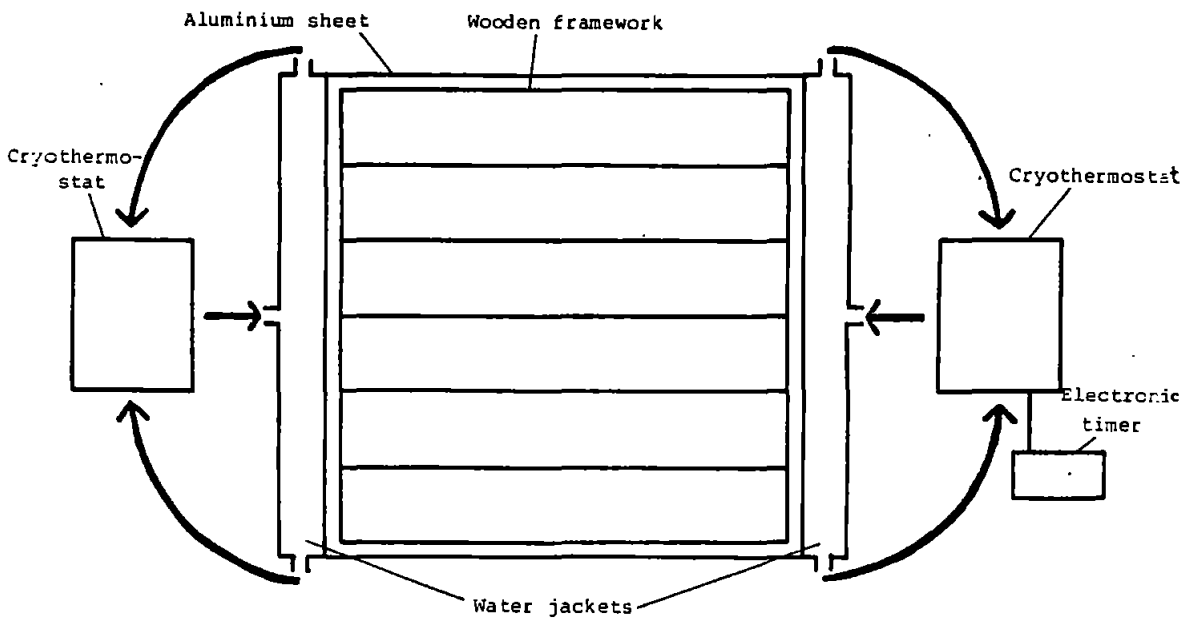
* He assured us that the thermogradient bars would never work efficiently ---- but he was proved wrong !!

Grooves were cut into the edges of the aluminium sheet which projected into the water - jacket in order to increase the surface area and therefore improve the rate of heat transfer from the water to the metal. The final dimensions of the sheet were 95×95cm and 0.5cm thick, 2.5cm at each end projecting into the water - jacket which was an aluminium pipe (3.5cm internal diameter) with a slot cut in the side into which the sheet was welded. The flow rate of the water was 18L /min.

Fig. 2.1

Top view of thermogradient bar.

Arrows represent direction of water flow.



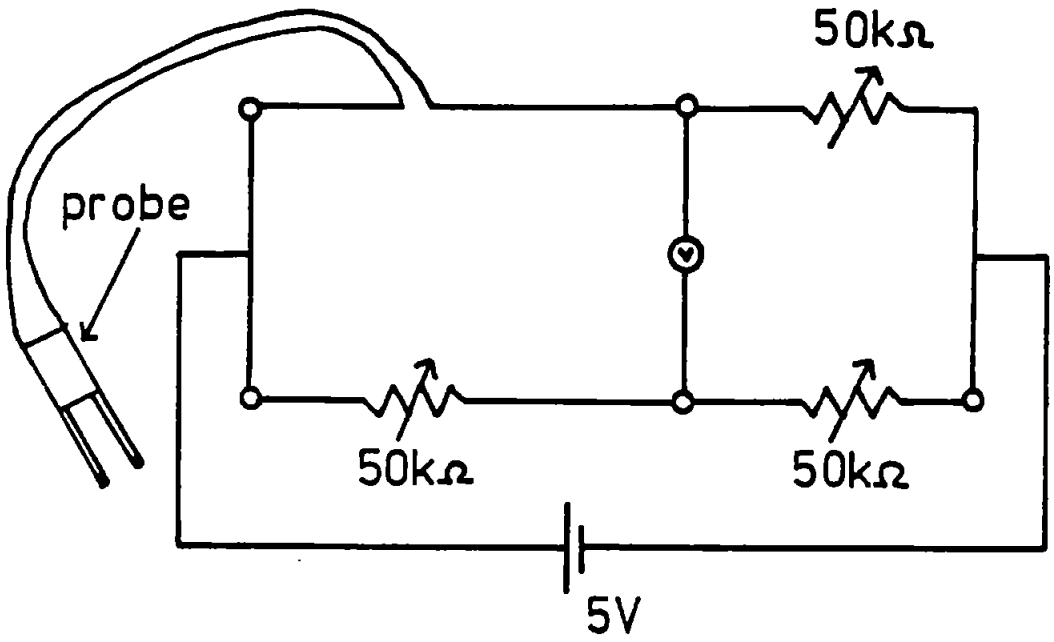
Control of soil moisture and aeration.

In order to prevent evaporation from the soil during experiments, each strip of soil on the bar, (i.e. each of the six replicates), was enclosed by an air - tight chamber. The chambers consisted of a wooden frame containing partitions which could easily be removed from the apparatus for cleaning purposes. The lid of each chamber was provided by either a perspex tank on the light thermogradient bar (Fig. 2.3), or a sheet of thick plate glass on the dark thermogradient bar. The most efficient seal between the wooden framework and the aluminium sheet was found to be a continuous strip of 'Blutack' which was frequently replaced. Strips of black, expanded rubber pipe insulation were glued onto the upper edge of the wooden framework to form the seal between it and the perspex tank or glass. When using the above arrangement there were few problems with soil drying out. During the course of an experiment soil moisture was checked regularly and extra water added if necessary to maintain the soil at field capaciy.

A special probe was designed to measure the soil moisture content. It measured the resistance to a flow of electrical current through the soil between two metal prongs which were a set distance apart (4mm.). The prongs were insulated at their ends to prevent discharge of current accross the aluminium sheet and were connected to a Wheatstone bridge(Fig.2.2). The meter was calibrated by measuring the soil moisture content of several samples by oven drying and relating this to the resistance shown by these samples using the probe. A new calibration curve had to be constructed when soil from a new site was used on the bars as the resistance was affected by soil type and compaction.The use of the probe was discontinued after a few experiments as the soil moisture content was sufficiently stable.

Fig. 2.2

Circuit diagram of the probe for measuring soil moisture



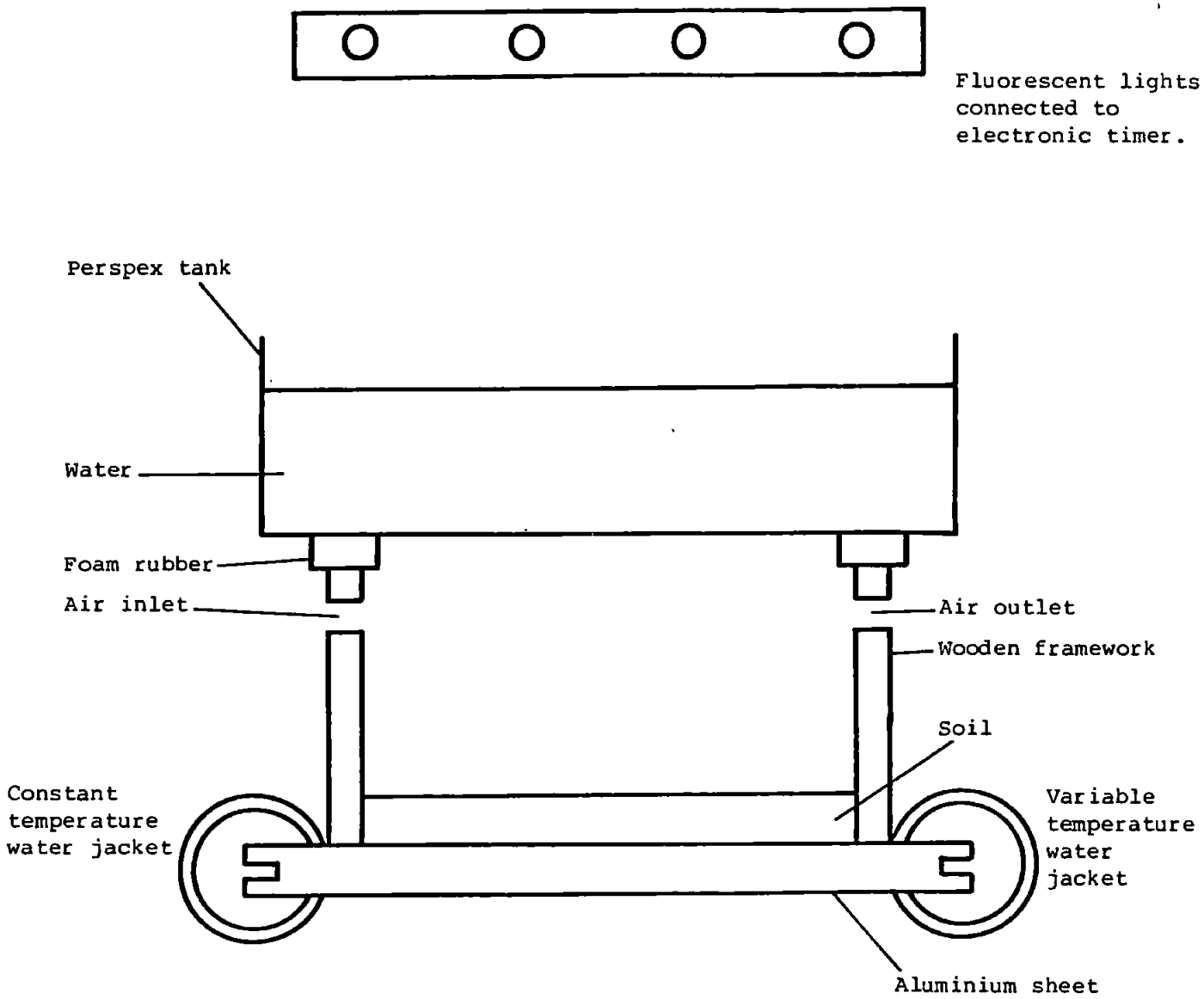


Fig. 2.3

Side view of thermogradient bar.

Aeration

Thompson (1977) found that, during experiments using harvested seeds in small enclosed chambers, aeration was necessary as the germination of some species was affected by lack of oxygen. However, the modified apparatus (Plates 1and 2) contained a large volume of air above the soil in each chamber, as the wooden partitions were 7cm high. Holes drilled at intervals near the top edge of each partition and in opposite corners of the outer framework, allowed sufficient passive movement of air to prevent oxygen depletion. When using the dark thermogradient bar, an additional slow flow of air was pumped through the chambers to help prevent ''damping off'' of the seedlings. To prevent the soil drying out the air was first saturated with water vapour by pumping it through a Dreschsel bottle containing water which stood in a water bath maintained at 12°c.The flow rate of air was slow in order to minimise its effect on the temperature gradient.

Lighting

Sufficient light was required to stimulate the germination of seeds contained in the layer of soil, (0.5cm deep), on the light thermogradient bar. Wooley and Stoller (1978) state that less than 1% of incident light penetrates further than 2.2mm in clay loam or sand unless the soil peds are greater than 1mm in diameter. Because of the techniques used here for preparing the soil many of the soil peds were 2-3mm in diameter and the soil maintained an open structure even after rewetting. The light is therefore likely to penetrate considerably further than is suggested by Wooley and Stoller. High fluence white light, (higher than 1.0 joule cm^{-2}), has been shown to inhibit seed germination (Mancinelli and Rabino 1978). So these two opposing factors had to be taken into account.

Lights giving a high red : far - red ratio in the spectrum were used as far - red light is known to enforce seed dormancy. The light was provided by a bank of four ''warm - white'' fluorescent tubes, suspended 40cm above the perspex tank which was filled with water to act as a heat filter. Light intensity at the soil surface was $98Wm^{-2}$. The lights were connected to an electronic time clock which could be programmed to give any predetermined photoperiod in a 24hour cycle.

Temperature control

This was provided by two cryothermostats (manufactured by 'Colora') with a working temperature range of -10°C to +100°C and a stability of ± 0.01°C, which pumped water around the water jackets. At the end of the aluminium sheet designed to give fluctuating temperatures the cryothermostat was modified so that the temperature of the water was controlled by an electronic thermostat with two variable temperature settings. The timing of alternations between the two temperature settings was controlled by means of the same programmable electronic timing device used for the lighting system.The water circulating around the other end of the sheet was maintained at a constant temperature.

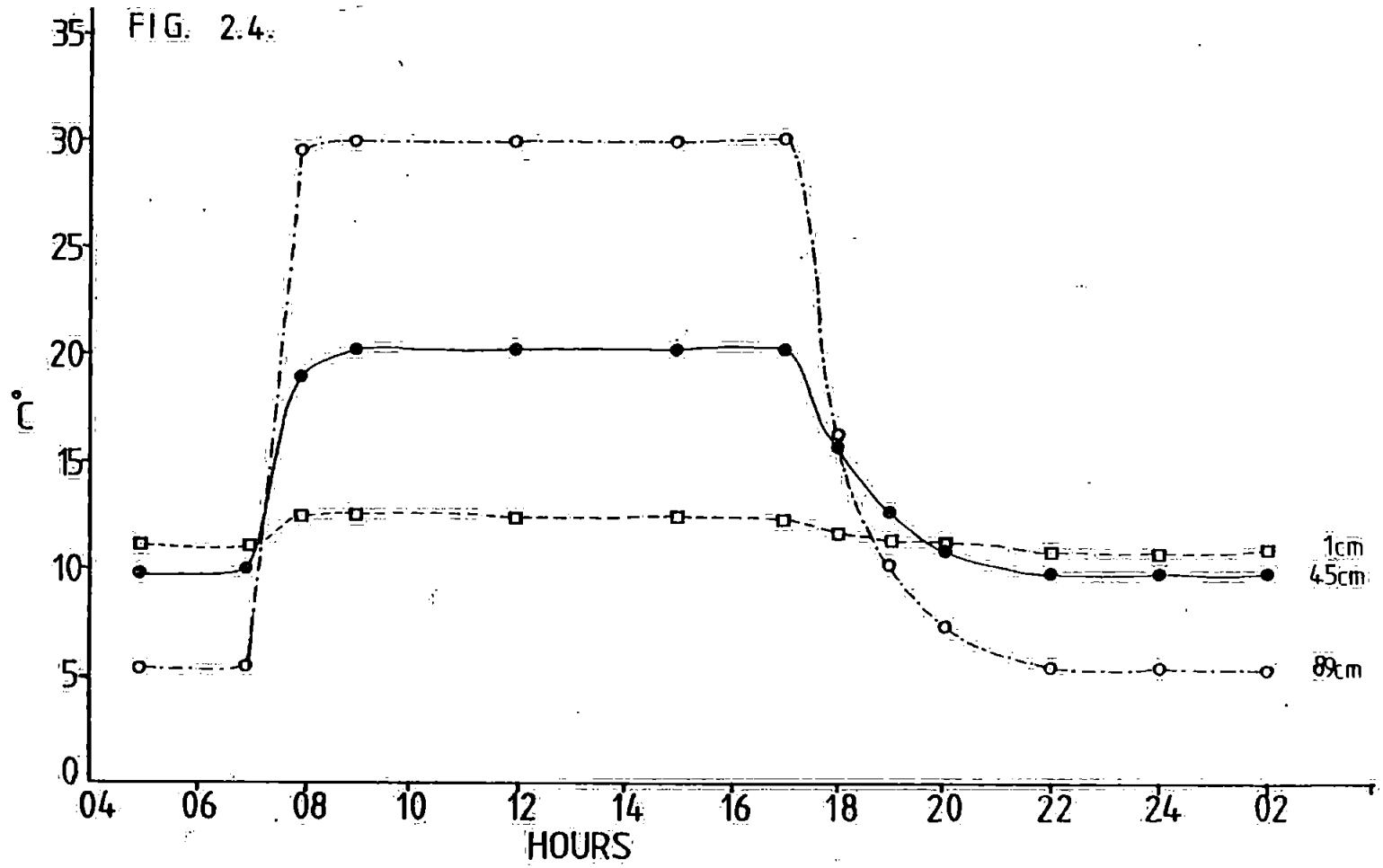
In order to achieve a uniform, stable temperature gradient across the aluminium sheet, the whole apparatus was well insulated. Each sheet was supported on thick glass - fibre insulation on top of a 7cm thick sheet of expanded polystyrene. All the pipes carrying water were covered in expanded rubber pipe insulation. Measurements of temperature along the bar (Figs.2.4 and 2.5), show that even at the 'constant' end soil normally experienced a small fluctuation in temperature (1.5°C).

FIG. 2.4. (Page 38).

Diurnal temperature changes recorded on the bar surface at 1cm(□), 45cm(●) and at 89cm(O) from the constant end of the bar.

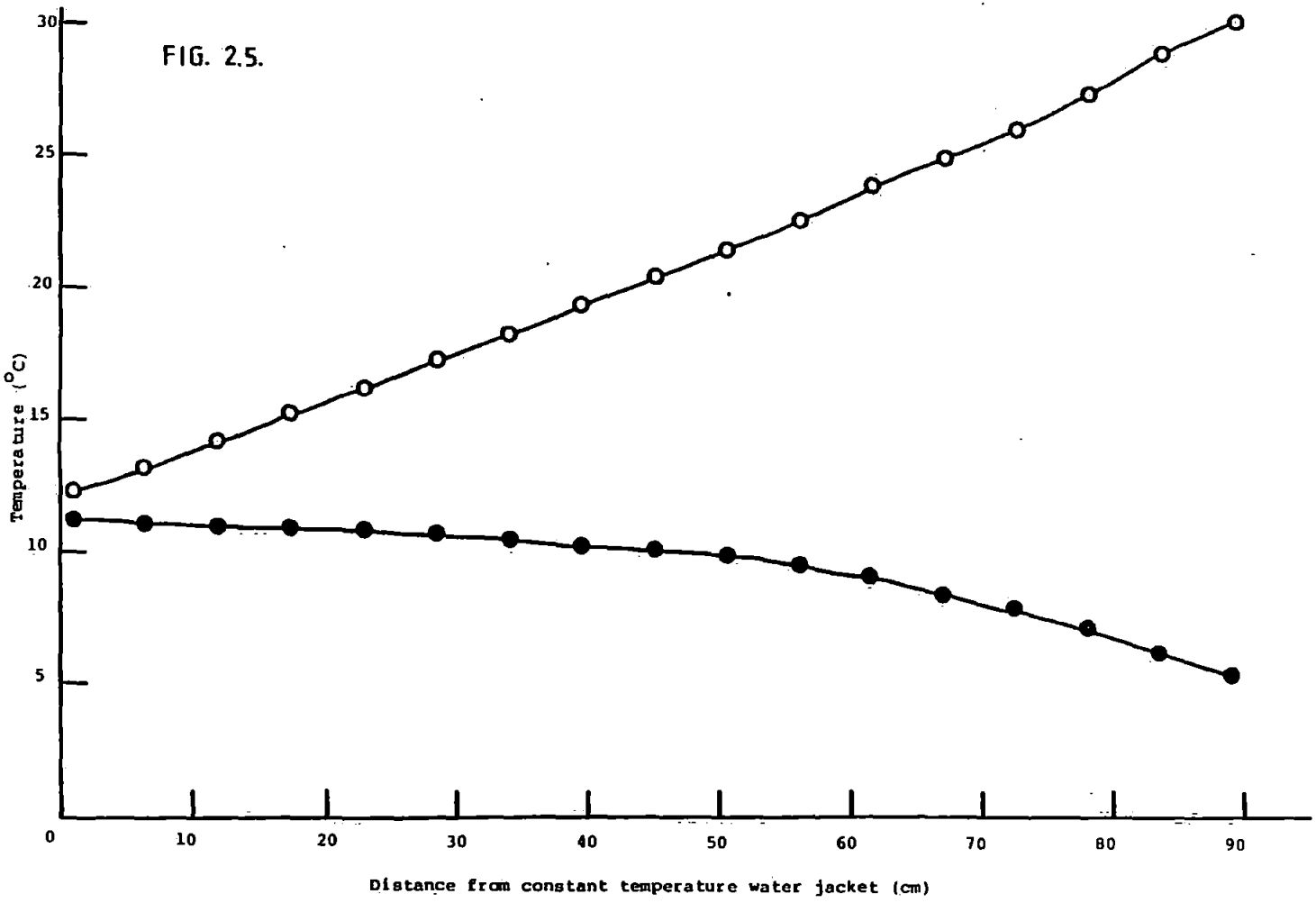
FIG. 2.5. (Page 39).

Temperature gradients at the bar surface during operation of the regime used in current experiments. Gradients maintained for 10 hours (O) and fourteen hours (●) in every 24. A ten hour dark period was included in the latter period.



Temperature changes during a 24hr. cycle

FIG. 2.5.


30
25
20
15
10
5
0
Temperature (°C)
10
20
30
40
50
60
70
80
90
Distance from constant temperature water jacket (cm)

Method of operation

Adjustments of the timing device and the thermostats provide for considerable flexibility with regard to the choice of temperature and light regime. The experiments described in this thesis used a regime in which the 'constant' temperature maintained at one end of the bar surface was 12°c. Different amplitudes of temperature fluctuation over the range 1.5°C - 25°C were induced by depressing the water temperature at the opposite end from 32°C to 4.8°C during a dark period of 10 hours (Figs. 2.4 and 2.5). This type of regime is similar to that experienced in the field in late spring.

In practice the apparatus was constructed so that two identical thermogradient bars were operated in parallel (see Plates 1 and 2). This allowed the simultaneous testing of soil samples in light and in darkness. The air temperatures in the light and dark chambers never differed by more than 2°C and this was insufficient to alter the soil temperatures, which were therefore identical on the two bars. The second bar was positioned 25cm vertically below the first and was thus out of direct illumination. The diffuse light reaching the soil surface on this bar was therefore only $4.5 Wm^{-2}$. Soil to be tested in darkness was spread on this bar and was covered by a layer of sterile sand (2-3mm) which prevented light from reaching the seeds in the soil. The reasons for using this technique are discussed in the section on 'preparation of soil samples'.

Collection of soil samples

Soil used on the thermogradient bars produced useful data if it had the following characteristics :

a) The number of seedlings of each species that emerged from a layer of prepared soil, (technique described below) one metre square and 0.5cm deep, exceeded 300.

b) It had not been disturbed for several months before the samples were collected.

Sites with a high density of viable seeds in the seed bank were therefore identified by trial germination tests in seed trays.

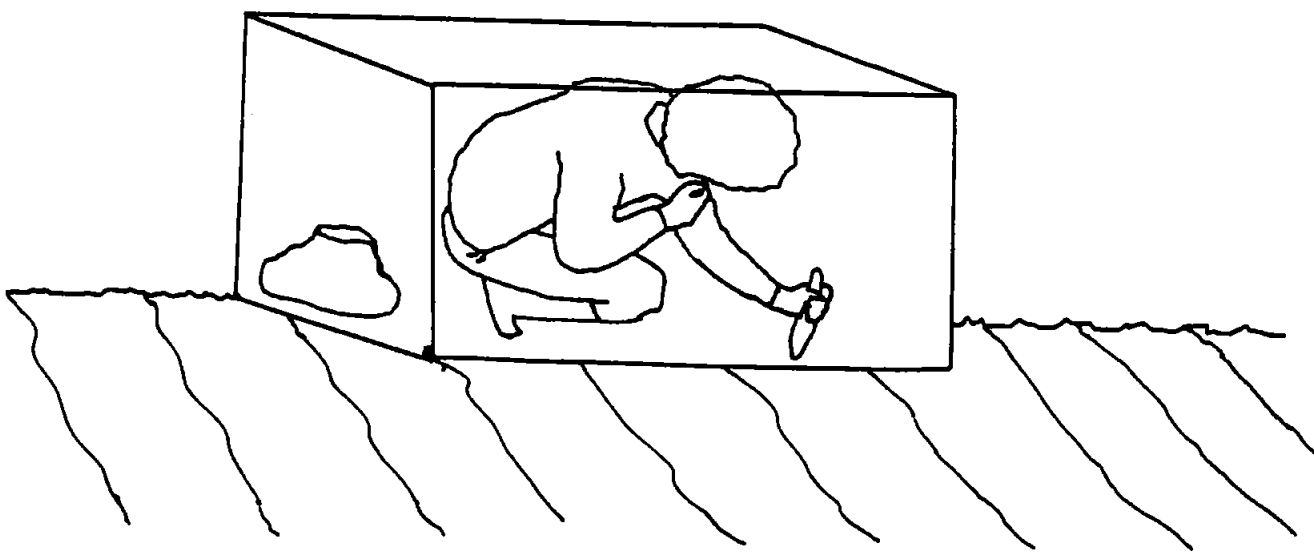
Plant seeds in the soil are generally aggregated about mother plants (Major and Pyott 1966). Aggregated populations are sampled most efficiently by taking a large number of soil cores and then mixing them. The depth distribution of seeds in soils is usually unknown but Chippindale and Milton (1934) showed approximately logarithmic decreases of numbers of seeds with depth in the soil and Rabotnov (1958) stated that 64 —99.6% of all pasture seeds studied were found in the 0-10cm layer of soil.

The sampling technique had to be such that the soil was collected in darkness as the aim was to maintain buried seeds in their origional dormancy states, at the time of collection, and then study their germination requirements under controlled conditions in the laboratory. Several different techniques for collecting soil samples in darkness were tried in the field, most of which were far too time consuming due to the large volume of soil needed for use on the bars at any one time. The only feasible method of sampling was based on the idea of working in a light-proof tent.

A 'black box' was therefore constructed using light weight tubular steel. Its internal dimensions were 100 X 90 X 72cm and it was covered on five sides with two layers of closely woven black material to prevent light penetration. It was placed over the surface of the soil to be sampled, and black P.V.C. bin-liners were filled with soil which was removed at intervals from the area covered by the black box(see Fig. 2.6). The box was moved as necessary to different areas within the sampling site. The P.V.C. bags were sealed before removing the black box and exposing them to light.

Fig. 2.6

Method of collecting soil samples in darkness.



It is possible that the act of collecting soil samples, though carried out in the dark, would alter the seeds' environment by changing the soil atmosphere. However, Karrsen (1981a) suggests that the oxygen and carbon dioxide content of the soil atmosphere is not likely to be very different from the air above the soil. He measured the levels of these gases in the soil atmosphere near batches of seeds buried at a depth of 10cm and found that oxygen did not show levels below 19% and carbon dioxide did not exeed 0.5% during a year of sampling. Coverage of the soil surface and penetration of roots did not influence the carbon dioxide level at a depth of 10cm. Holm (1972) has shown that volatile components such as acetone can influence buried seeds but this is only likey to be a significant effect in compacted soil or at relatively great depths. The soil collected for use on the thermogradient bars was taken from the top 7cm of the soil profile which was generally well aerated.

Preparation of soil samples

Ideally, to maintain the seeds in their natural state, the wet soil should have been placed directly onto the thermogradient bars for testing. This was not possible as the soil samples had to be thoroughly mixed and stones, roots and vegetation removed. The soil could then be spread on the thermogradient bars in a uniform layer 0.5cm thick. The soil was, therefore, dried rapidly in the dark, mixed and passed through a 3mm sieve. Some soils did not contain sufficient seeds to provide a statistically significant number of germinations in the dark. Seeds in these soil samples to be tested in the dark were concentrated by passage through a second sieve to remove the <300μm soil fraction.

It has been shown that seeds containing less than 6% moisture are

insensitive to red light, due to inactivation of the phytochrome (Berrie, Paterson and West 1974, Kendrick 1976). The above manipulations of the dry soil were therefore carried out safely in dim light as the seeds were 'fixed' with respect to the action of light. When on the bars the soil was rewet to field capacity with a fine mist sprayer in order to avoid disturbing the surface.

There is clearly a possibility that the drying process may have altered the dormancy state of the seeds in the soil. However the surface layers of soil dry out to a considerable degree during dry spells even in Britain (Benjamin 1974 and data mentioned in this thesis). It seems likely that buried seeds experience at least partial drying and rewetting under field conditions. There is some reason to believe, therefore, that the artificial drying carried out here did not depart from the normal experience of many buried seeds.

A further factor which may have affected seed dormancy was abrasion of the seed coats when excavating soil samples and in their preparation by seiving. A controlled experiment, conducted by Wesson and Wareing (1969(a)) to investigate this effect, showed that there was no significant effect on seedling emergence in the dark.

Seeds kept moist at low temperatures ('stratification') or held in dry storage, may undergo various changes in the temperature limits for germination (Vegis 1964; Roberts and Lockett 1977). The time period between collecting soil samples and rewetting on the thermogradient bar was therefore kept to a minimum.

Initial experiments in which the entire dark thermogradient bar was maintained in complete darkness for fourteen days produced severely etiolated seedlings and also encouraged ''damping - off'' disease. It was not possible to identify these seedlings. A layer of sterile sand (2-3mm) was subsequently spread over the surface of the

soil and the sand surface exposed to dim white light ($4.5Wm^{-2}$). This enabled the seeds to remain in darkness but seedlings could grow through the sand into the light thus reducing etiolation. The presence of sand also facilitated the initial rewetting procedure as it was no longer necessary to do this in total darkness.

Controlled experiments in growth cabinets have shown that there is no significant difference between the numbers of seedlings emerging from bare soil in total darkness and soil covered with 2-3mm sand in dim light. This shows that insufficient light penetrated the sand to trigger germination and suggests that the sand does not affect the soil atmosphere or soil moisture significantly.

Other uses for the thermogradient apparatus

The thermogradient bar apparatus was also used to test harvested seeds. Seeds were placed on the surface of a layer of sterile compost, which acted as a water reservoir, on the thermogradient bars. Seeds to be tested in the dark were covered with a layer of sand, as described for the naturally buried seeds.The results of these experiments are described in Chapter 3. The apparatus has many other potential uses, some of which are described in the 'Further applications' section in this chapter.

Data collection

The optimum time for running an experiment on the thermogradient bars before plotting the total number of seedlings emerged was found to be fourteen days for most species.There was only a 3-4% increase in total numbers after a further seven days, and this increase occurred uniformly accross the bars.

In order to express the distribution of seedling emergence

graphically the six chambers formed by the wooden partitions were divided into five sections, each with the same increment in the number of degrees of temperature fluctuation experienced. The area of each section was calculated using the graph shown in Fig.2.5 and each section was assigned a mean amplitude of temperature fluctuation. Since the sections were of unequal area, the numbers of seedlings were expressed on a per unit area basis.The data from some experiments in the light were adjusted to take account of the increasd concentration of seeds in the soil on the dark bar due to additional sieving.

Sufficient seedlings were found to enable statistically significant conclusions to be drawn in a total of fourteen species. The results from these species are shown and discussed in Chapters 3,4,5 and 6.

Statistical analysis of data

Valid statistical comparisons could only be made using data from soil samples collected at a particular site on the same sampling date, In individual tests, where all the seedlings arise from the same seed population, the total number of seedlings per unit area in each section of soil on the thermogradient bars can be compared. Other comparisons were made using data expressed as a percentage of the total number of seedlings that emerged in each section or in the soil sample as a whole. To investigate the effect of differences in the mean amplitude of temperature fluctuations in each section, while taking account of random variation between the replicate treatments, the analysis of variance technique was used.

Any statistical treatment involving percentage data was carried out on the values transformed by the arc-sine method as described by Bishop (1966). The transformed value was θ, in degrees, when $\sin\theta =$

$\sqrt{(x/100)}$, in which x was the percentage to be transformed.

To measure the significance of differences between treatments, a t-test was carried out on pairs of mean treatment values. Calculations were reduced by producing a figure for the L.S.D. between mean values, from the data presented in the relevant analysis of variance table.

Validity of data

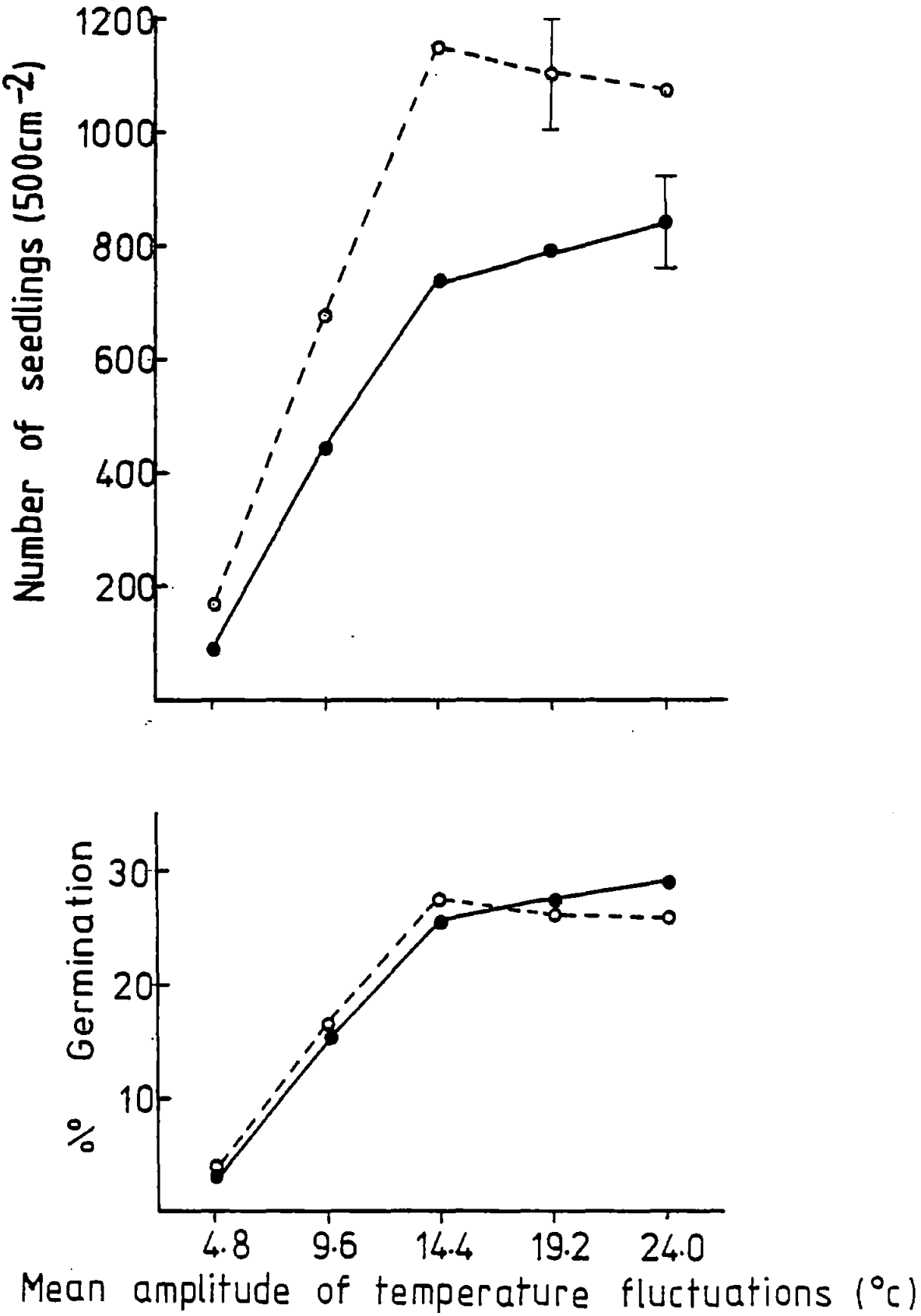
It was thought that the temperature response data obtained from the thermogradient apparatus may be very variable, due to variation in the soil sampling and preparation techniques. This was tested by taking two separate sets of soil samples from the same site, within a few days of each other.The samples were then tested separately on the thermogradient bars in the dark. The temperature response curves of Coronopus didymus, which was contained in the soil, were compared. If the response curves were similar it could be assumed that the techniques were repeatable from test to test.Differences in the response of a particular species on different sampling dates could then be ascribed to real differences in the dormancy states of the seeds rather than random variation included in the experimental procedure.

FIG. 2.7.

The response of naturally buried Coronopus didymus seeds to diurnal temperature fluctuations in darkness. Seeds were collected from the same site on 20-10-81 (●) and on 29-10-81 (○).

Vertical bars represent the least significant difference between treatment means (P = 0.05).

FIG.2.7. Coronopus didymus



The results show that there was no significant difference between the shape of the response curves (Fig. 2.7). However, there was a very large difference in the total number of seedlings emerging from the two soil samples. This was to be expected as the horizontal distribution of buried seeds in the soil is very variable (Major and Pyott 1966). The soil sample containing more Coronopus didymus seeds must, at some stage, have supported more parent plants producing 'patches' of seeds than the other soil sample.

When assessing the seedling emergence figures it would have been useful to have an estimate of the number of viable seeds of each species contained in the soil sample. Unfortunately, seed separation is a long, laborious procedure involving wet sieving and floatation techniques (Roberts and Ricketts 1979) and it is not easy to assess seed viability accurately. Regretfully therefore, determination of the total viable seed load of the soils tested was not attempted.

Results from the light thermogradient bar

It is possible that any apparent stimulation of germination by fluctuating temperatures on the light thermogradient bar is not a real effect, but an artefact due to limited light penetration into the layer of soil.If the need for fluctuating temperatures is abolished in seeds exposed to the light, one would expect a constant level of seedling emergence at all amplitudes of temperature fluctuation. The effect of this, combined with limited light penetration into the soil, would be to superimpose a constant level of seedling emergence due to light on a variable level caused by the stimulation of germination by fluctuating temperatures in that portion of the soil below the level of light penetration.

The very different shapes of the temperature response curves of

several species in the light and dark (e.g. Epilobium tetragonum (Fig.5.4) and Juncus acutifolius (Fig.5.5) render this explanation unlikely. However the effect of soil on altering the light quality (in particular the red/far-red ratio) cannot be ignored entirely (Frankland and Poo 1981) and this may affect the germination responses of more deeply buried seeds.

Effects of the temperature regime

Like soil depth, the temperature regime employed on the bars (Fig.2.5) represents a compromise between conflicting requirements. Ideally one would like to be able to account for differences in germination along the bars purely in terms of fluctuating temperatures. In practice seeds in different positions on the bars experience differences in (a) temperature fluctuation, (b) extremes of temperature and (c) mean temperature. Points (a) and (b) are discussed fully in Chapter 5, but (c) can be dealt with more briefly here. It is theoretically possible to arrange the temperature regime on the bars in such a way that the mean temperature experienced by the seeds does not vary. Reference to Fig.2.5 shows that this can be achieved either by raising the 'constant' temperature or by lowering the lower temperature experienced at large fluctuations. In fact neither of these changes is desirable. Raising the constant temperature would result in the smaller fluctuations occurring at temperatures far higher than are ever associated with such fluctuations in the field. Measurements in the field indicate that small fluctuations are always found at low absolute temperatures. Lowering the lower extreme temperature would reduce it to 0°c or below, which would either prevent germination altogether or kill germinating seedlings.

Using the regime adopted (FIg.2.5) the mean temperature experienced by the sections at opposite ends of the bars varies from 13.6°c to 20°c. From the data presented in Grime et. al. (1981) it seems possible that 13.6°c is just below the constant temperature at which a small minority of the species tested could be expected to germinate to a high percentage. For two reasons, however, it is doubtful whether this low mean temperature actually did affect germination. Firstly a significant part of each day was spent at the higher temperature. Secondly, and more importantly, even the section of the bar nearest the constant end experienced a mean temperature fluctuation of 4.8°c, and it is well known that the lower temperature limit for germination can be extended downwards quite a long way by relatively small temperature fluctuations (Grime et. al. 1981 , Thompson and Grime 1983). We therefore suggest that the variation in mean temperature along the bar probably has no practical effect on germination.

Further applications of the thermogradient apparatus

All the data described in this thesis were obtained from seeds tested at a particular temperature and light regime which approximated to that measured at different depths in bare soil in the field in late spring. The apparatus is suited to extending these experiments by altering the temperature and light regime to that found at other times of the year or in soil beneath different types of vegetation or litter layers. The length of time for which harvested seeds are exposed to the maximum temperature in each diurnal cycle is known to be important in governing the rate of induction or alleviation of secondary dormancy (Totterdell and Roberts 1979) and this could be investigated for buried seeds by altering the electronic time-clock.

It has been noted that using soil containing the naturally buried reserve of seeds presents a problem in that the total number of viable seeds in a specific volume of soil is not known and germination figures cannot be expressed as a percentage of the number of viable seeds in that sample. This could be overcome by using sterile soil or other growth media into which known numbers of seeds have been introduced, allowing more precise statements to be made about the germination percentages associated with particular treatments.

The potential for comparison of harvested and naturally buried seeds is much greater than has actually been realised in the study reported here (Chapter 3). In particular it should be possible to apply particular pre-treatments to harvested seeds (e.g. far-red light) in an attempt to duplicate the behaviour of buried seeds. Harvested seeds are not affected by the cyclic changes in dormancy state induced by seasonal changes in absolute temperatures in the field (Karssen 1981(b)) and a preliminary study on how these changes affect the response of buried seeds to fluctuating temperatures is described in Chapter 4. The apparatus could be used to study this in a more controlled way by burying samples of seeds in trays of soil in the field and then testing them at regular intervals throughout the year on the thermogradient bar apparatus.

Laboratory experiments have been carried out on harvested weed seeds which indicate that stimuli which affect secondary dormancy often act synergistically (Vincent and Roberts 1977, Roberts and Benjamin 1979). Buried seeds are likely to respond in a similar way and this could be investigated using the thermogradient apparatus. Soil could be chilled on the bars before being exposed to a fluctuating temperature regime. Interactions between temperature and nitrate could be studied by artificially altering the nitrate

concentration of samples of soil taken from the same site on a particular date. Soil atmosphere may also affect the germination response of buried seeds (Karssen 1981(b)) and this could be altered by pumping air of varying composition through the chambers.

CHAPTER 3

COMPARISON OF THE GERMINATION RESPONSES OF HARVESTED AND BURIED SEEDS

Introduction

Both freshly harvested and buried seeds are likely to be dormant. The seeds of a vast majority of species have a period of innate dormancy which is imposed on the seeds as they mature on the mother plant. Such dormancy prevents the seed from germinating viviparously and usually remains for sometime after the ripe seed is shed or harvested. Sometimes after a seed has lost its innate dormancy, secondary dormancy may be induced. This is usually the result of seeds being supplied with water in an environment where some other factor is unfavourable for germination. It has been suggested that, in buried seeds, secondary dormancy may be induced by adverse temperatures, limited oxygen supply, volatile inhibitors or prolonged darkness (Karssen 1981(b), Wesson and Wareing 1969(b)). The dormancy thus induced may persist for a long time after the inhibitory factor has been removed. The buried seeds may also be subject to enforced dormancy which is imposed by some limitation in the environment, such as darkness. In this case it is removed immediately when the seeds are exposed at the soil surface.

Most investigations of seed dormancy are carried out on harvested seeds which have been stored dry, at various temperatures, before testing. Although both harvested and buried seeds are dormant, there is growing evidence that the results of laboratory germination tests cannot be used to interpret the germination responses of buried seeds in the field with any accuracy. This may be due to seasonal changes in the dormancy states of buried seeds caused by changes in the absolute temperatures which are experienced at different times of the year. There may also be more general differences in response to dormancy breaking stimuli such as light and fluctuating temperatures, caused by differences between the innate and secondary dormancy

imposed on the seed (Karssen 1982).

The bulk of this thesis is concerned with the germination responses of naturally buried seeds to a particular range of diurnal temperature fluctuations in both light and darkness. However, in an initial investigation, the responses of naturally buried and harvested seeds from the same site were compared for four species ---- Holcus lanatus, Rumex obtusifolius, Epilobium tetragonum and Juncus acutiflorus.

Materials and method

The seeds of Holcus lanatus, Rumex obtusifolius and Epilobium tetragonum and the soil containing buried seeds of these species were collected from sites near Plymouth Polytechnic Experimental Station, Rumleigh (Nat.Grid Ref. SX 443 679). Seeds and soil containing Juncus acutiflorus were collected from a marshy area of Warleigh Woods,Devon (Nat. Grid Ref. SX 449 608). Photographs of these sites can be seen on Plates 4.1 and 4.2.

The harvested seeds were dried at room temperature and removed from their covering structures by gentle abrasion.The unwanted material was separated from the seeds by using an apparatus designed at the Unit of Comparative Plant Ecology, Sheffield University.The apparatus is illustrated on Plate 3 (back of thesis). The seeds are collected in a separate chamber from the seed coats by blowing air at a controlled velocity, depending on the size and weight of the seeds, through a chamber containing the mixture of wanted and unwanted material. Design details of this apparatus can be obtained from the author.

The cleaned seeds were stored in the dark at 5°c for several months until needed.

Germination tests

The soil was collected from the field and prepared a few days bofore it was tested on the thermogradient bars. The soil sampling and preparation technique is described in Chapter 2.

The temperature regime used on the bars for testing soil samples and seeds was such that the 'constant' temperature maintained at one end of the bar surface was 12°c. Different amplitudes of temperature fluctuations over the range 1.5°c - 25°c were induced by depressing the bar temperature at the opposite end from 31°c to 6°c .

This regime is shown in Figs. 2.4 and 2.5 and is similar to that experienced in the field in surface layers of soil in late spring.

Harvested seeds to be tested on the thermogradient bars were placed on the surface of a layer of sterile compost which acted as a water reservoir. In order to test seeds in darkness a layer of sterile sand (2-3mm thick) was spread over the soil containing seeds, (or the seeds themselves) and the sand surface exposed only to dim white light. Epilobium tetragonum and Juncus acutiflorus were tested only in the light.

All the germination tests were continued for fourteen days before the number of seedlings was counted. Emergence was defined as the appearance of the plumule above the surface of the soil or sand.

Analysis of data

Each germination test contained three replicate strips of soil or compost on the thermogradient bars. The replicates were divided into five sections, each with the same increment in the number of degrees of temperature fluctuation experienced. The five sections were each assigned a mean amplitude of temperature fluctuation and,since they

were of unequal area, the numbers of seedlings were expressed on a per unit area basis ($500 cm^{-2}$).

The number of seedlings emerging from the harvested seed samples was not directly comparable with the number emerging from buried seeds of the same species. This was because the total number of viable seeds in the soil was unknown and was not likely to be equal to the number of harvested seeds placed on the surface of the compost. The mean number of seedlings in each section was therefore expressed as a percentage of the maximum mean number in a section for each germination test. The section in which the greatest number of seedlings emerged was designated 100% emergence for convenience but there were obviously still several seeds which had not germinated and may have been viable in these sections. The results are illustrated graphically on the following pages. The transformation of the data to percentages enabled the shape of the temperature response curves to be compared for harvested and buried seeds. However, it conceals the possibility that different proportions of buried and harvested seeds may remain viable but ungerminated under the temperature regime used here.

Results and discussion

In all the germination tests, apart from that for harvested Epilobium tetragonum (Fig. 3.3), the seeds showed considerably greater germination at large amplitudes of temperature fluctuations than at small amplitudes. However, in some tests, the number of seedlings emerging was reduced when the mean amplitude of temperature fluctuations exceeded 19.2°c. This was particularly marked in buried Holcus lanatus seeds tested in the dark (Fig. 3.2), when seedling emergence dropped by over 30%.

The harvested seeds of Holcus lanatus, Rumex obtusifolius and Epilobium tetragonum showed considerably more germination at low amplitudes of temperature fluctuations than buried seeds. For example, in the light test, buried Rumex obtusifolius seeds showed 20% germination at 4.8°c fluctuation but harvested seeds showed 70% germination (Fig. 3.1). The germination responses of harvested and buried seeds of the above species were very similar at amplitudes of temperature fluctuation above 9.6°c.

FIGS. 3.1, 3.2 and 3.3.

The response of naturally buried (——) and harvested (----) seeds to diurnal temperature fluctuations.

Seeds were tested in the light (O) and in darkness (●).

FIG. 3.1. Rumex obtusifolius

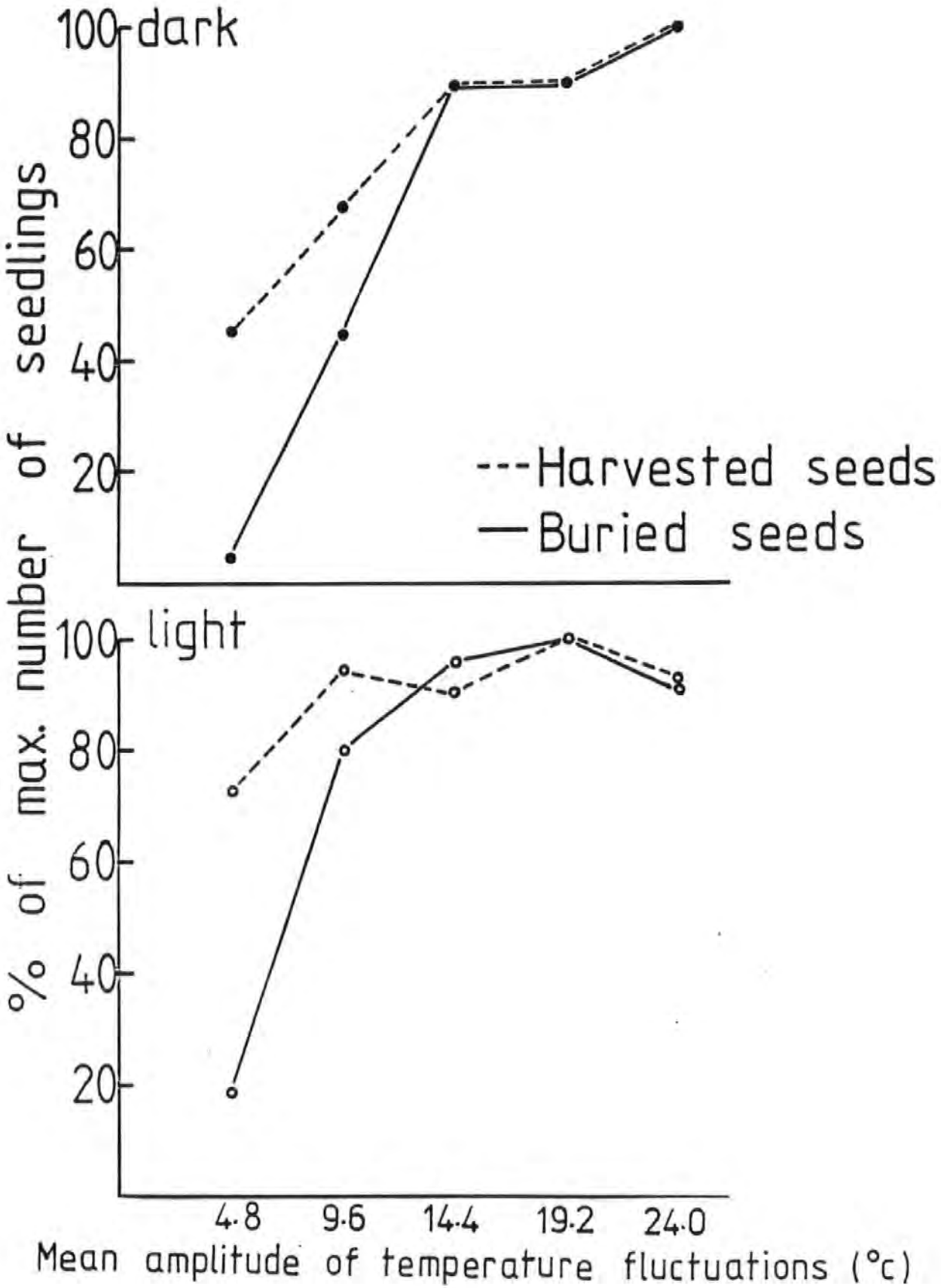


FIG. 3.2. Holcus lanatus

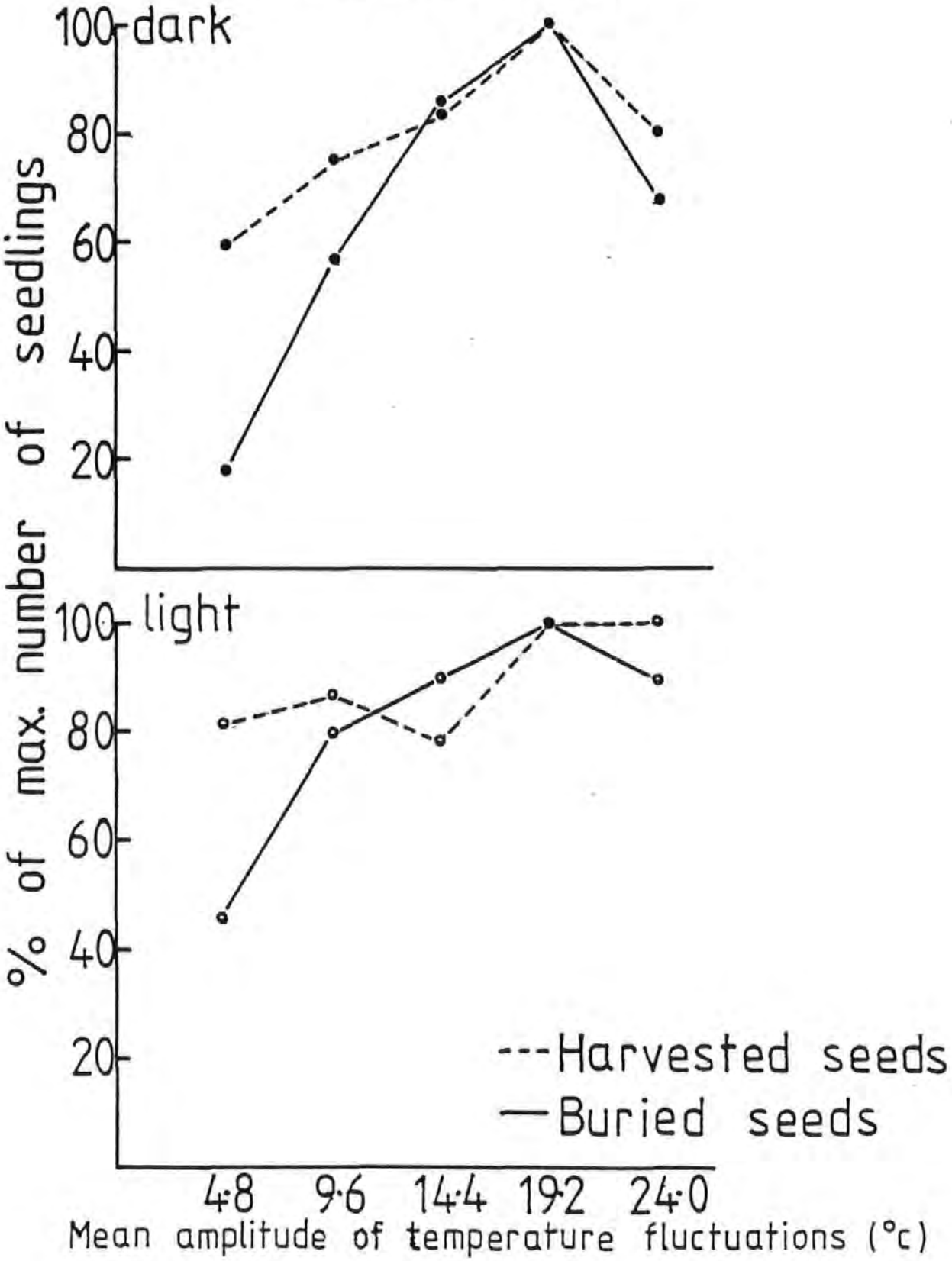
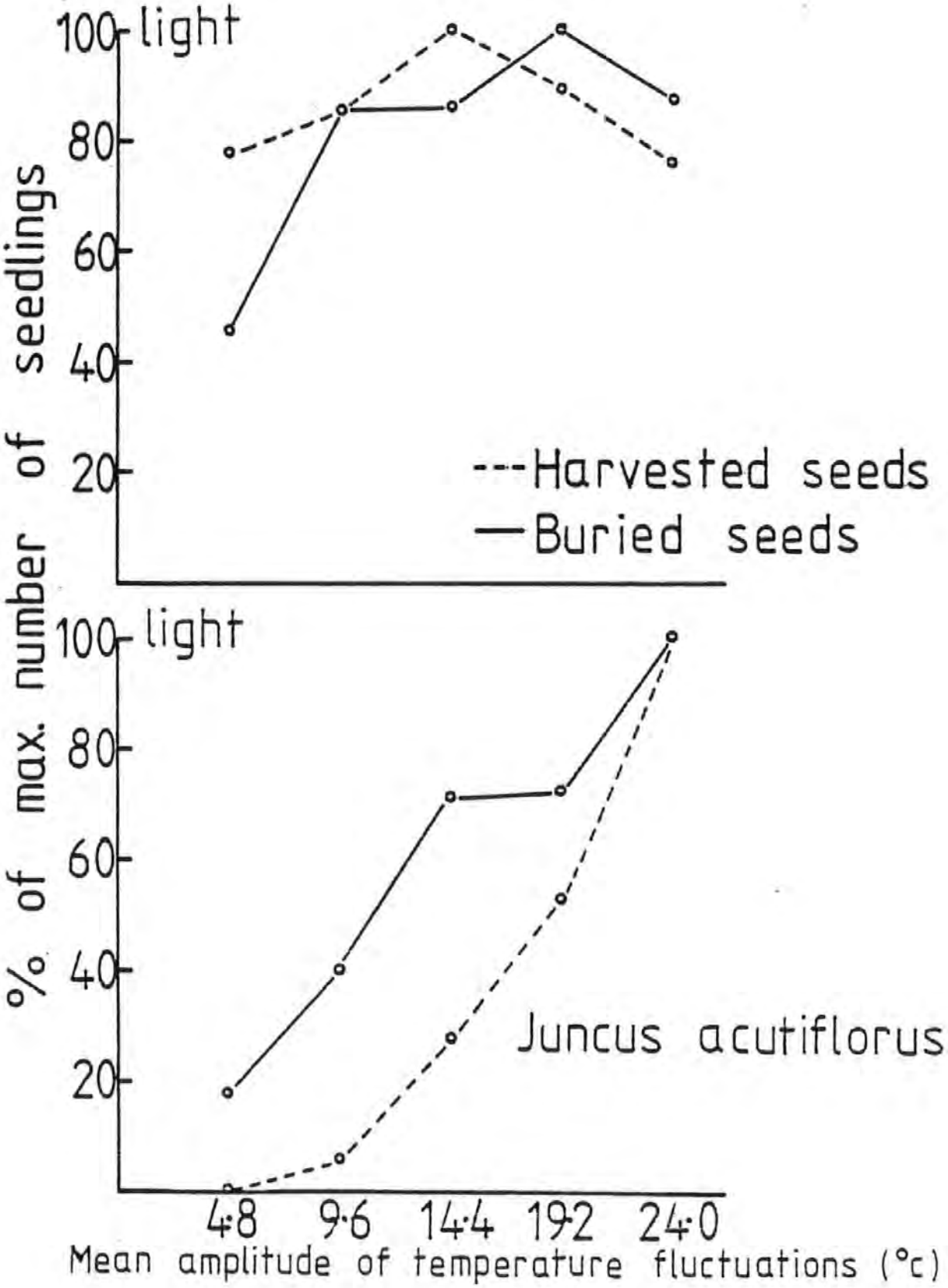


FIG. 3.3. Epilobium tetragonum



Juncus acutiflorus (Fig. 3.3) was an exception to the above findings as buried seeds germinated to a greater extent at low amplitudes of temperature fluctuations than the harvested seeds. For both buried and harvested seeds of this species it seemed likely that a further increase in the amplitude of temperature fluctuation above 24°c, the maximum employed here, would have resulted in a further increase in germination.

The shapes of the response curves of Holcus lanatus and Rumex obtusifolius to increasing amplitudes of temperature fluctuations were different in the dark and in the light. The main difference was a narrower optimum temperature range for germination in the dark. The requirement for fluctuating temperatures was abolished to a large extent in harvested seeds of Holcus lanatus, Rumex obtusifolius and Epilobium tetragonum in the light. This is in agreement with the results of Thompson and Grime (1983). In tests with buried seeds, light could not substitute for fluctuating temperatures to the same extent. It therefore seems that during burial, seeds may acquire not only a requirement for light (Wesson and Wareing 1969(b)), but also a requirement for fluctuating temperatures.

It is obvious from these results that harvested seeds do respond differently from buried seeds to a fluctuating temperature regime. One explanation may be that dry storage of the harvested seeds caused a reduction in dormancy. However, this process (after-ripening) that occurs during storage is temperature dependent and is prevented or greatly reduced by low temperatures. The seeds used in these tests were stored at 5°c. Storage of Rumex crispus seeds for five years at 2-4°c resulted in no appreciable change in dormancy (Cavers 1974).On the other hand Rumex obtusifolius was somewhat affected by storage at 1.5°c for nine months (Totterdell and Roberts 1979).

It is unlikely that after-ripening could account entirely for the differences in temperature response between buried and harvested seeds. An alternative explanation, mentioned above, is that either a requirement for temperature fluctuations may be acquired, or the amplitude of fluctuation required initially may be increased, during burial.Many factors experienced during and after burial of seeds in soil are different to those experienced during dry storage of seeds in the laboratory and may affect their dormancy state. A change in dormancy state may then affect the subsequent response to fluctuating temperatures.

The simplest explanation for the changes noted above is that a secondary dormancy is induced during burial when the seeds remain imbibed in darkness for long periods.This secondary dormancy then enforces a requirement for fluctuating temperatures before germination can take place. Secondary dormancy has been found to occur when light-requiring seeds are held imbibed in darkness for several days. It is called skotodormancy and its development is temperature dependent (Taylorson and Hendricks 1973).An experiment was therefore devised to compare the germination percentages of harvested seeds, before and after imbibed dark storage, with those after dry dark storage at the same temperatures. This experiment and others using harvested Rumex obtusifolius seeds are described in Chapter 7.

CHAPTER 4

SEASONAL CHANGES IN DORMANCY OF BURIED SEEDS

Introduction

In Chapter 1 reference was made to investigations of seasonal patterns in buried seed dormancy ----- this subject has recently been reviewed by Karssen (1982). Seasonal periodicity in environmental conditions and in the dormancy of buried seeds have been found and it was concluded that germination only occurs in the field if the range of internal requirements of the seed overlap with the range of actual conditions in the habitat.

These findings suggest that naturally buried seeds contained in soil samples from the same site but collected on different dates may respond differently to the temperature and light regimes experienced on the thermogradient bars. The temperature limits for germination are widest when seeds are least dormant (Vegis 1964, Baskin and Baskin 1980). Buried seeds of summer and winter annuals pass through distinct patterns of change in dormancy which show a phase difference of half a year (Karssen 1981 (a)). It is possible that other species which emerge in flushes throughout the year (e.g. Stellaria media) do not show these patterns.

As was mentioned in Chapter 1, fluctuations in the field behaviour of seeds are governed by changes in the two components of the system : 'environment' and 'seed' (Fig. 1.1). This chapter describes investigations of the seasonal changes in both components. Changes in the germination responses of six species of naturally buried seeds to a given temperature regime on the thermogradient bars and changes in certain environmental variables (absolute temperatures,amplitude of diurnal temperature fluctuations and rainfall) were studied. The emergence of seedlings in response to these environmental variables was recorded in the field and under more controlled conditions on the roof of Plymouth Polytechnic.

Sites from which soil was collected

Soil used in this investigation was collected from four different sites, all near Plymouth Polytechnic Experimental Station, Rumleigh (Nat. Grid Ref. SX 443 679). These sites will subsequently be referred to by their site numbers given below.

Site 1

Old vegetable plot at the Experimental Station, Rumleigh.The ground remained fallow during the course of the investigation and was largely bare with some cover by patches of weeds. Soil contained large numbers of seeds of Epilobium tetragonum and Coronopus didymus.

Site 2 (Photograph plate 4.1)

Derelict pasture that had previously been used for market gardening. Continuous vegetation cover mainly consisting of clumps of Holcus lanatus and Rumex obtusifolius. The soil contained large numbers of seeds of Holcus lanatus, Rumex obtusifolius, Coronopus didymus and patches of Cardamine hirsuta.

Site 3 (Photograph plate 4.2)

Field containing a daffodil crop. The soil contained large numbers of seeds of Epilobium tetragonum,Polygonum aviculare, Coronopus didymus and Cardamine hirsuta.

Site 4 (Photograph plate 4.3)

Field containing a potato crop. The soil contained large numbers of seeds of Stellaria media, Spergula arvensis and Chenopodium album.

Plates 4.1, 4.2 and 4.3.

Top photograph on each page shows the site from which soil was collected. The vegetation at the soil surface is shown below.

Plate 4.1

Site 2. Derelict pasture.

Plate 4.2.

Site 3. Field containing daffodil crop.

Plate 4.3.

Site 4. Field containing potato crop.

Germination tests

Soil was collected from the above sites at intervals throughout the year and tested on the thermogradient bars. The soil sampling and preparation technique is described in Chapter 2.

The temperature regime used on the bars for testing soil samples was such that the 'constant' temperature maintained at one end of the bar surface was 12°C and different amplitudes of temperature fluctuations over the range 1.5°C-25°C were induced by depressing the bar temperature at the opposite end from 31°C to 6°C. A diurnal photoperiod of 14 hours was maintained throughout the experiments. This regime is shown in Fig.4.1. Initial tests on the dark thermogradient bar were unproductive as seedlings were frequently etiolated or suffered from "damping-off" and could not be identified. When an effective technique had been established to produce reliable results (see Chapter 2) there was insufficient time to investigate seasonal changes in the germination responses of seeds in the dark. The only species for which data are available is Coronopus didymus.

All the germination tests were continued for fourteen days before the number of seedlings was counted. Emergence was defined as the appearance of the plumule above the surface of the soil.

Analysis of data

The main aim was to gain a general impression of the extent to which the germination response to a fluctuating temperature regime for six species of buried seeds, varied between sampling dates. This information was used to determine the validity of comparing the germination responses of different species when the soil containing the seeds was collected on different dates. The data were therefore illustrated in a way that enabled intraspecific variation in

temperature response (i.e. between sampling dates for a particular species), to be compared with interspecific variation (i.e. between the six species studied). It was not hoped to collect sufficient data for any one species to build up a clear picture of seasonal dormancy changes that could be correlated with seasonal changes in environmental variables.

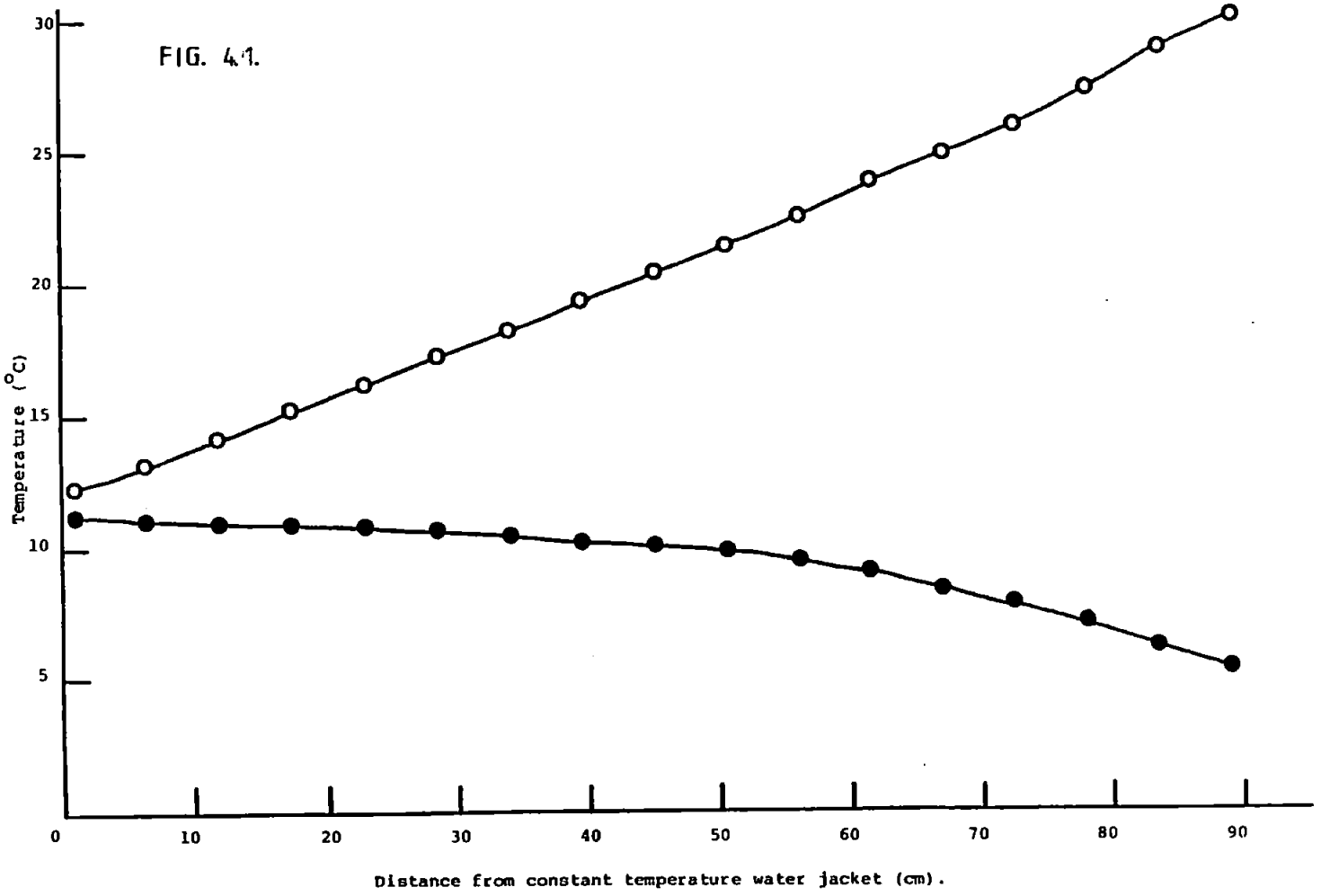
In order to express the distribution of seedling emergence graphically the six chambers (replicate treatments) formed by the partitions on the thermogradient bar were divided into five sections, each with the same increment in the number of degrees of temperature fluctuation experienced. The area of each section was calculated using Fig.4.1 and each section was assigned a mean amplitude of temperature fluctuation. Since the sections were of unequal area the number of seedlings was expressed on a per unit area basis. In order to compare the seedling emergence patterns on the bars for each species on the different sampling dates the mean number of seedlings emerging in each section was expressed as a percentage of the mean total number in each replicate.

Absolute numbers of seedlings could not be compared between sampling dates with much confidence due to the uneven distribution of seeds in the soil across any sampling site. However, these numbers have been included in the graphs to give an idea of the number of seedlings on which each curve is based. The mean numbers of seedlings that emerged in each section in individual tests were compared statistically using the analysis of variance technique. Where there was a statistically significant difference between mean seedling numbers ($p=0.05$) this is indicated by an asterisk on the relevant graph.

FIG. 4.1.

Temperature gradients at the bar surface during operation of the regime used in the experiments described here. Gradients maintained for ten hours (O) and fourteen hours (●) in every 24. A ten hour dark period was included in the latter period.

FIG. 41.


30
25
20
15
10
5
Temperature (°C)
0
10
20
30
40
50
60
70
80
90
Distance from constant temperature water jacket (cm).

FIGS. 4.2 - 4.8.

The response of naturally buried seeds from soil collected on different dates to diurnal temperature fluctuations in the light (O) or darkness (●).

Asterisks indicate that the response curve is significantly different from a straight line (p=0.05).

Total numbers of seedlings are given above each point on the curve.

FIG. 4.2

Cardamine hirsuta

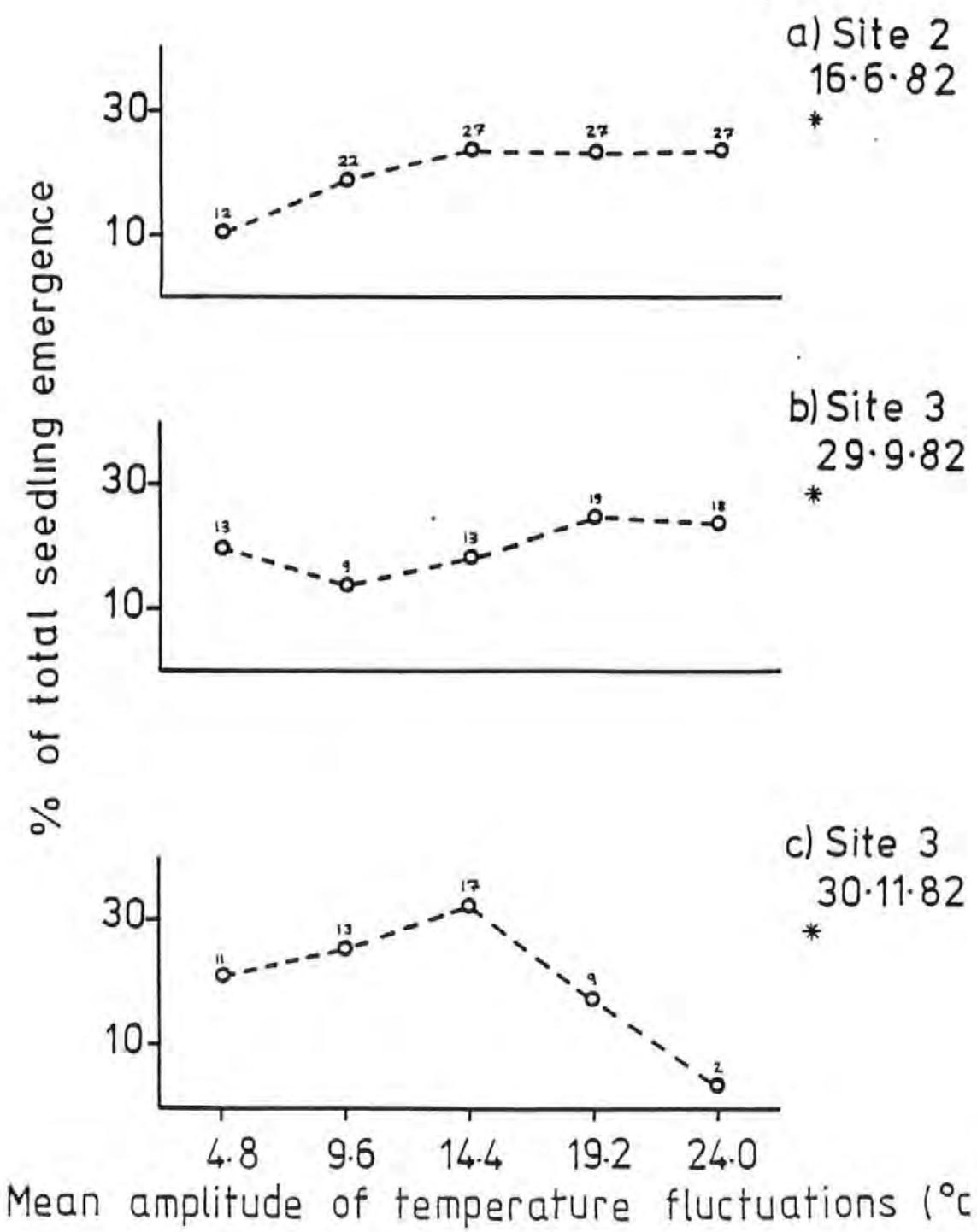


FIG. 4.3 Coronopus didymus (light)

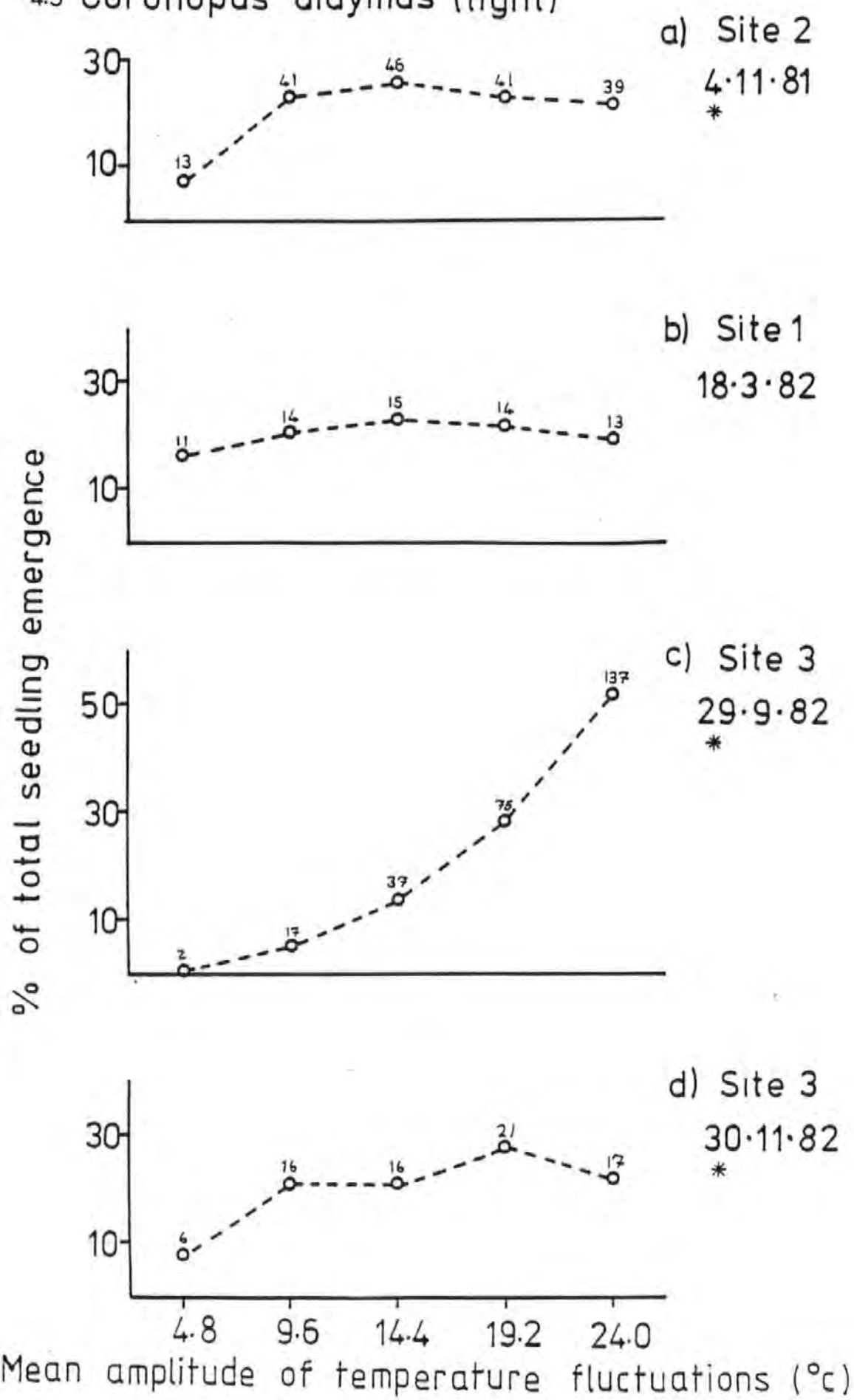


FIG. 4.4 Coronopus didymus (dark)

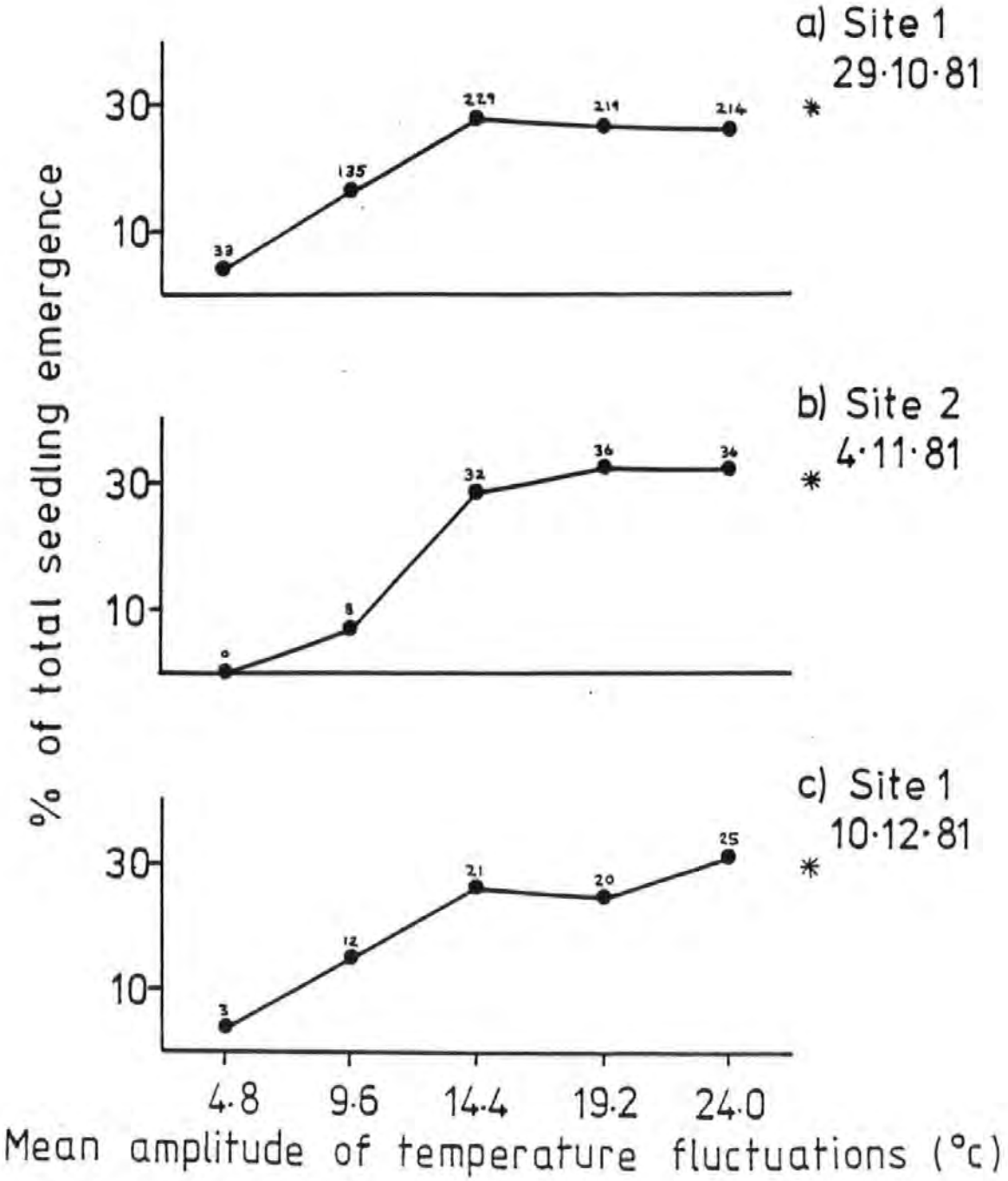


FIG. 4.5 Epilobium tetragonum

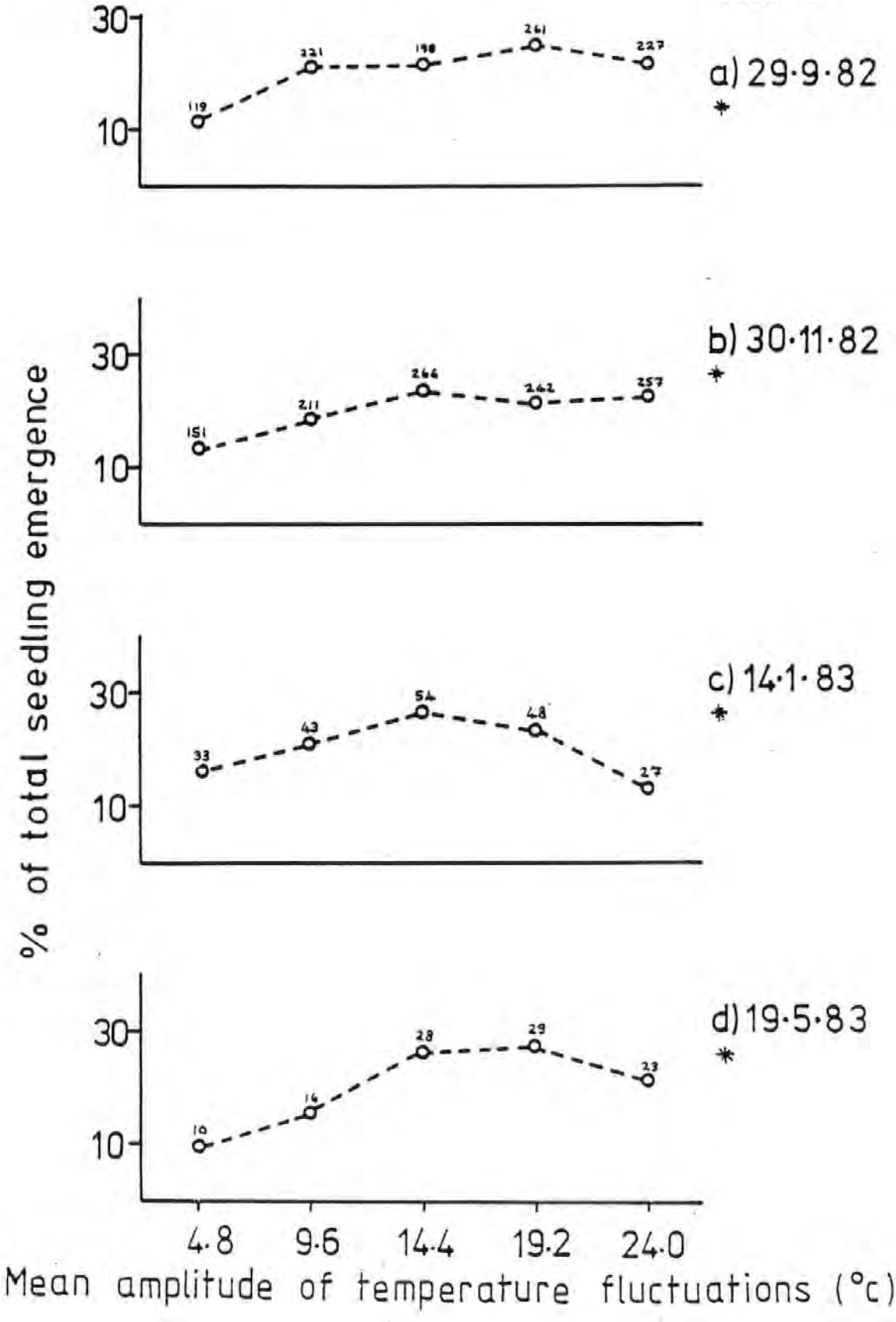


FIG. 4.6

Polygonum aviculare

All from Site 3

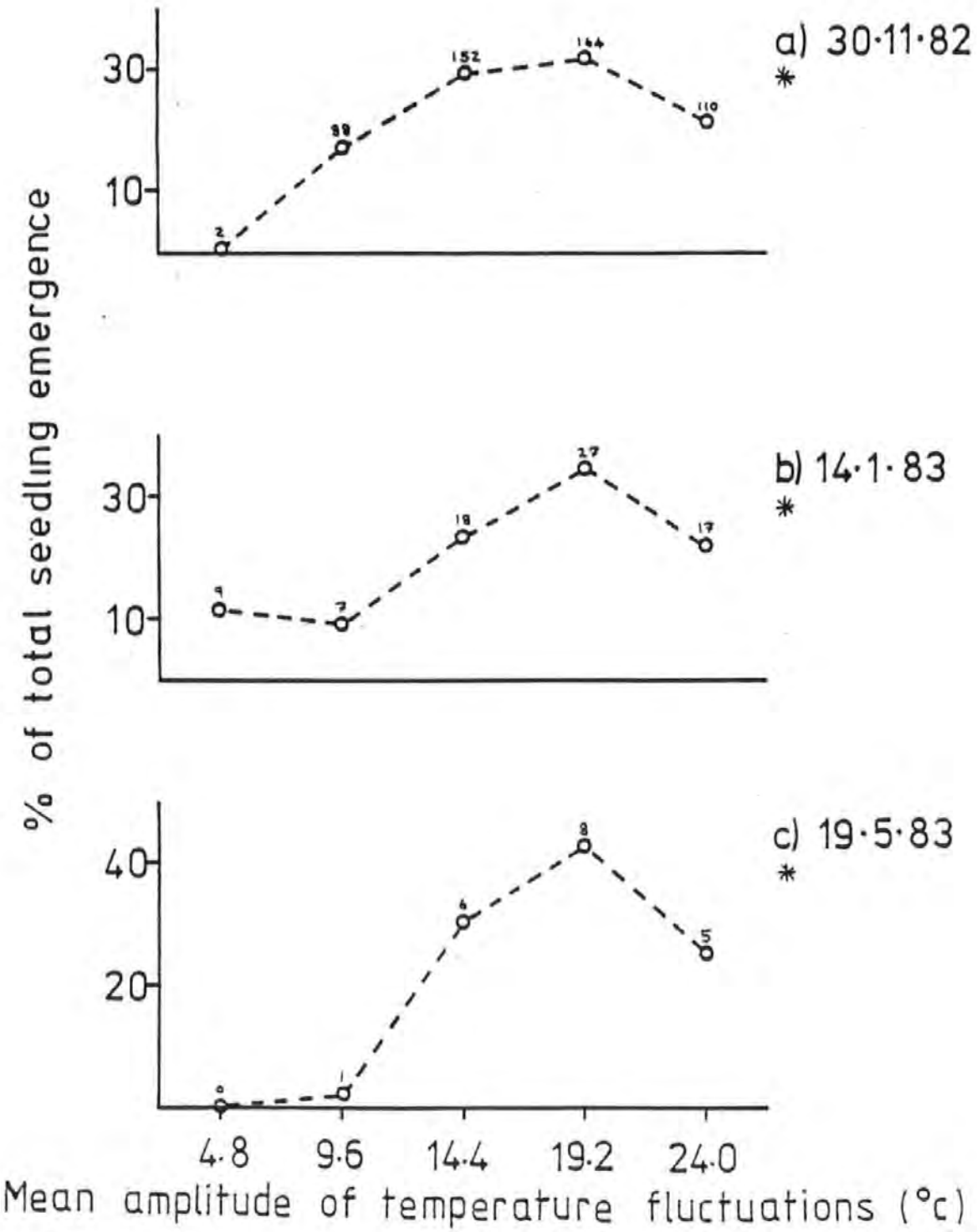


FIG. 4.7 Spergula arvensis

All from Site 4

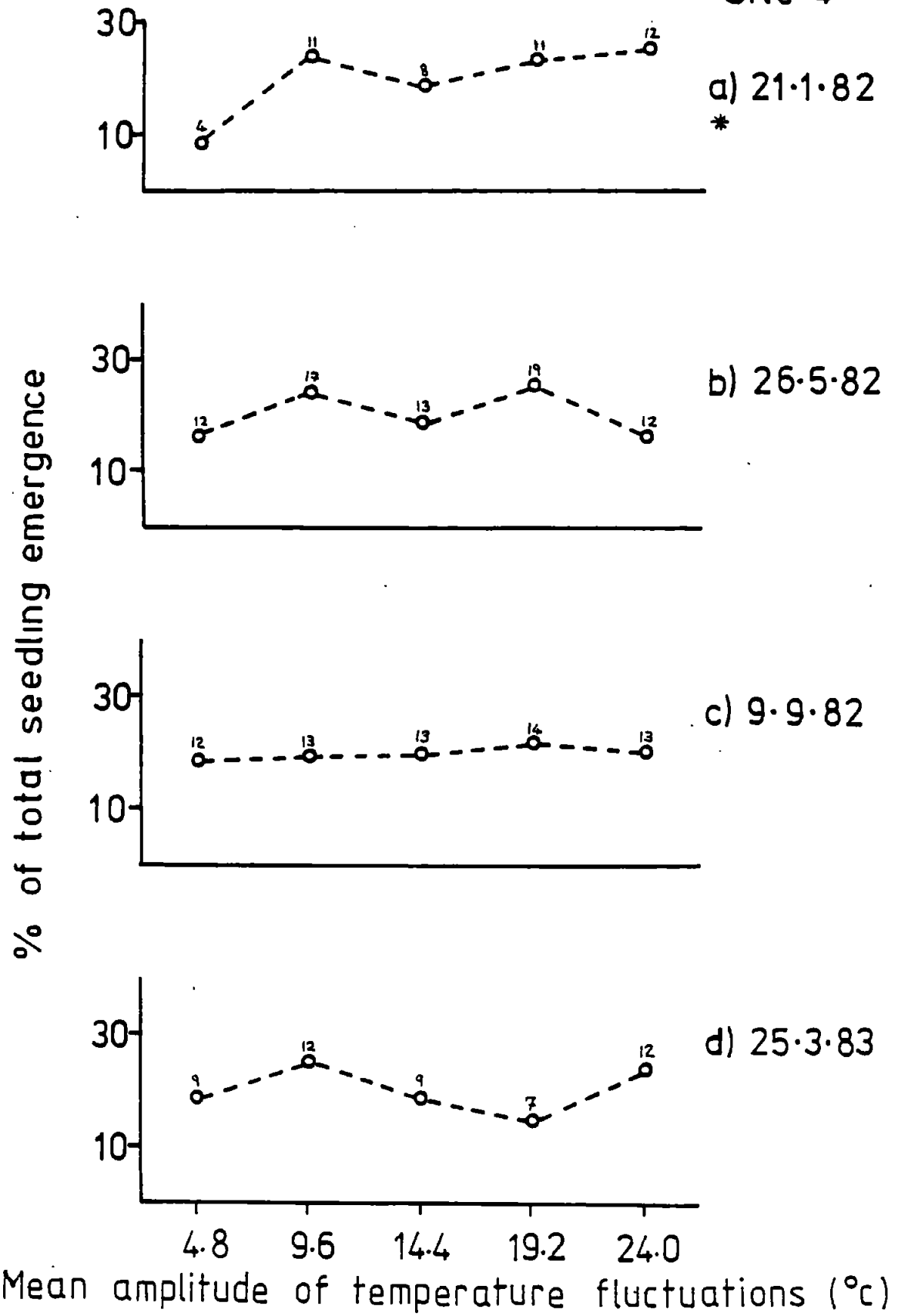
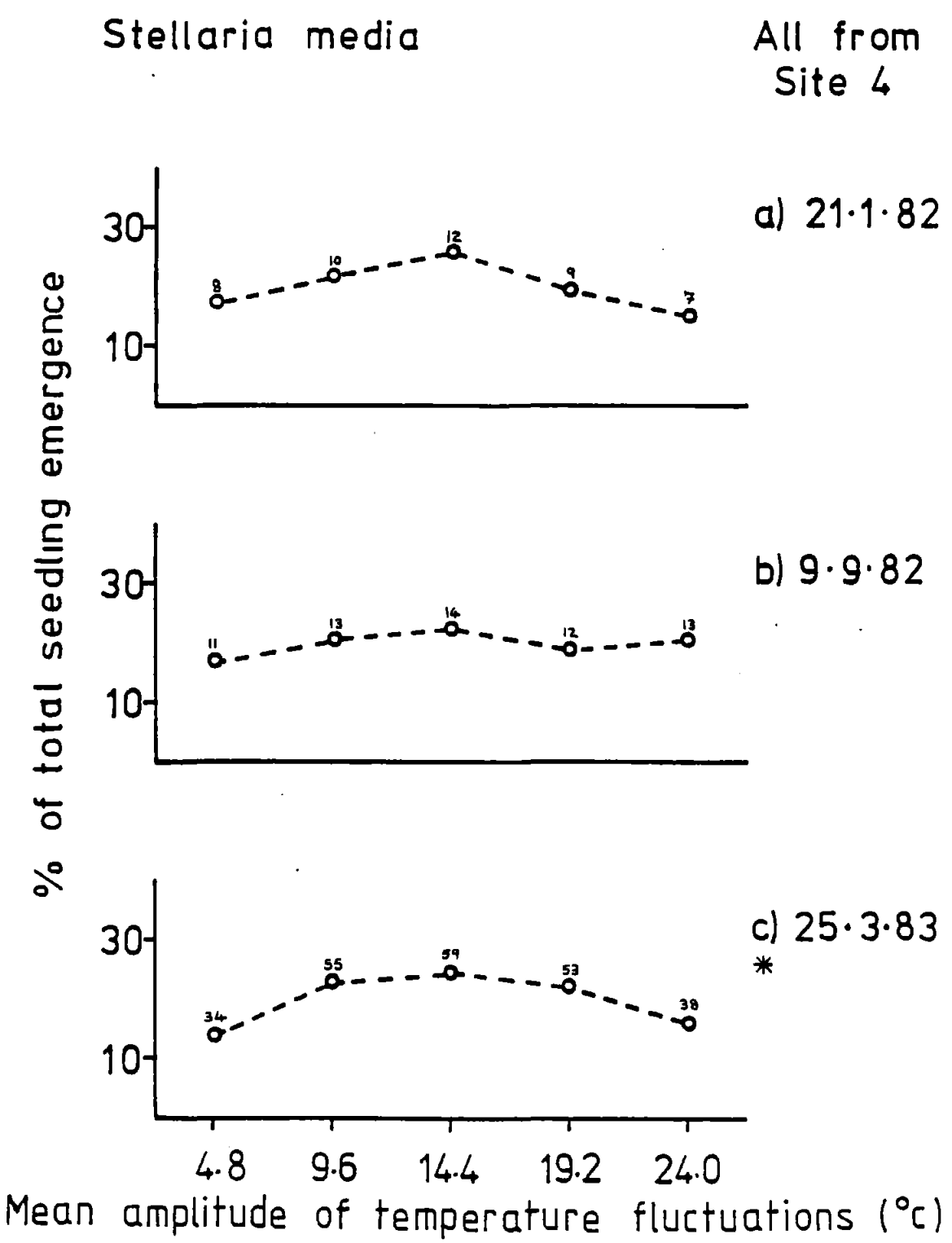


FIG. 4.8


Stellaria media
All from Site 4
a) 21·1·82
b) 9·9·82
c) 25·3·83
*
% of total seedling emergence
30
10
8
10
12
9
7
11
13
14
12
13
34
55
59
53
38
4·8
9·6
14·4
19·2
24·0
Mean amplitude of temperature fluctuations (°c)

Curves on graphs without an asterisk are not significantly different from a horizontal straight line and therefore seedling emergence was not affected by the changes in temperature regime in the different sections. The seedling emergence patterns illustrated by curves with an asterisk may have been caused by a response to the different amplitudes of temperature fluctuations or alternatively by different absolute temperatures. Some temperatures may stimulate loss of dormancy whereas others may enforce secondary dormancy. However absolute temperatures which are known to enforce dormancy in several species of harvested seeds, i.e.>28°C or <8°C, (Grime et. al. 1981) are only experienced at the extreme ends of the thermogradient bars. It is therefore likely that the shape of the response curves is largely due to the effects of different amplitudes of temperature fluctuations. This is discussed in more detail in Chapter 5.

The response curves indicate the relative importance of different amplitudes of temperature fluctuations in breaking buried seed dormancy at different times of the year. If the graphs for a certain species show a fairly constant response curve (e.g. Polygonum aviculare, Fig. 4.6) then the relative stimulatory effect of different amplitudes of temperature fluctuations remains constant through the year but very different total numbers of seedlings may emerge at a particular site at different times of the year. After the test on soil collected on 19-5-83 large numbers of ungerminated seeds were noticed but this was not the case for soil collected on 14-1-83 from the same site. Thus different proportions of the seeds in the seed bank may be in a state of 'true' dormancy where they will not germinate under any circumstances while the rest of the seeds remain responsive to temperature fluctuations.

Results and Discussion

Summarising the results shown in Figs. 4.2-4.8, it seems that the response curves of most species remain fairly constant throughout the year, with the apparent exception of Coronopus didymus and Cardamine hirsuta.

Other work has shown seasonal changes in the germination response of buried seeds to a particular temperature regime (Karssen 1981, 1982, Baskin and Baskin 1980). Karssen (1982) suggested that these changes were likely to be caused by seasonal changes in absolute temperatures which would affect the dormancy state of buried seeds according to the species (e.g. summer or winter annuals react differently to high summer temperatures). As buried seeds pass through seasonal cycles of imposition and alleviation of secondary dormancy different proportions of the population will be germinable and this is reflected in the great changes in the total number of seedlings emerging on the thermogradient bars on different sampling dates (e.g. compare Polygonum aviculare collected on 30-11-82 and 19-5-83, Fig.4.6). These results suggest that at least in some species dormancy is an 'all or nothing' phenomenon and, although the proportion of dormant seeds may show great seasonal variation, the response of the non-dormant fraction of the population to temperature fluctuations is always similar.

In general the seeds showed considerably greater germination at large amplitudes of temperature fluctuations than at small amplitudes. This is in general agreement with harvested seeds of the same species (Thompson and Grime, 1983).

There are distinct differences in the shape of the temperature response curves between species. Both Spergula arvensis and Stellaria media (Figs. 4.7 and 4.8) show little response to temperature

fluctuations at any time of the year. This agrees with the erratic flushes of seedlings appearing throughout the year which have been recorded for Spergula arvensis (Lawson, Waister and Stephens 1974) and Stellaria media (Roberts and Feast 1970, Roberts and Potter 1980).

The temperature response curves for Epilobium tetragonum (Fig.4.5) generally show a gradual increase in seedling numbers with increasing temperature fluctuations up to approximately 14°c. There is a narrower optimum range of temperature fluctuations and also much lower total numbers of seedlings germinating in January and towards the end of May than in the autumn. The absolute numbers of seedlings recorded suggest that a large proportion of the buried seed population is in a state of true dormancy (i.e. germination is prevented whatever the external conditions) at certain times of the year.

Unlike Epilobium tetragonum, seeds of Polygonum aviculare have a much narrower range of optimum temperature fluctuations for germination which remains fairly constant throughout the year but is narrowest in May. The optimum amplitude for temperature fluctuations is between approximately 14-19°C which would restrict seedling emergence to fairly large gaps but reduce the numbers emerging on completely bare ground. The total numbers of seedlings were greatly reduced in January and mid-May compared with November.

Courtney (1968) buried ripe Polygonum aviculare seeds near the soil surface and noted field emergence patterns. He found that low winter temperatures (2-4°C) were required to overcome innate dormancy and seedling emergence began in late February when soil temperatures started to rise. Ungerminated seeds removed from the field at the end of May had become dormant and it was presumed that this dormancy was induced by the higher temperatures of early summer. Justice (1941) showed that if non-dormant seeds of Polygonum aviculare were allowed

to become air dry at laboratory temperatures, dormancy developed. Drying-out of the soil might therefore reinforce the effect of higher temperatures in inducing dormancy. These observations are in agreement with the low numbers of seedlings that emerged on the thermogradient bars in mid-May. The increased numbers on the bars in November is again in agreement with Courtney (1968) who found that the induced dormancy persisted throughout the summer but was overcome during autumn and winter.

The emergence of Coronopus didymus on the thermogradient bars was very different in autumn from the remainder of the year. In the field Coronopus didymus flowers and sheds its seed in the late summer. The soil collected on 29-9-82 (Fig. 4.3) probably contained a large proportion of fresh seeds and the results suggest that these seeds have a requirement for large fluctuations which is gradually lost during burial. It is therefore probably not the example of a major seasonal change in response to fluctuations which it at first appears to be.

Soil collected on 4-11-81 was tested both in the dark (Fig. 4.4) and the light (Fig. 4.3). Comparing the two curves, it seems that buried Coronopus didymus seeds that have received no light require larger amplitudes of temperature fluctuations (> approximately 14°C) to break dormancy than seeds receiving a light stimulus at the same date. A similar conclusion was reached by Thompson and Grime (1983) for several species of harvested seeds tested in a fluctuating temperature regime in the light or darkness.

The emergence pattern of Cardamine hirsuta (Fig. 4.2) seems to be rather unusual as seedling emergence is greatly reduced by fluctuations of more than approximately 14°C in November. As this species is a winter annual, one would expect the range of temperatures

over which it can germinate to become wider as the summer progresses and to be almost irresponsive to temperature fluctuations in the autumn. This appears to be the case to a limited extent when the response curves of seeds collected on 16-6-82 and 29-9-82 are compared. The seeds appear to be readily germinable in mid-June so some factor other than temperature must prevent their emergence in the field. Newman (1963) found that a lack of long periods of continuous moisture in the field in summer months prevented germination of two winter annual species.

The response curve shown by seeds collected on 30-11-82 is difficult to explain. Perhaps by the end of November Cardamine hirsuta is re-entering dormancy and a sign of this is a reduction in the range of temperatures at which it will germinate, which appears as a reduction in germination at large fluctuations. If this is the correct explanation it is an example of very different behaviour from Polygonum aviculare.

Field experiments

It seems that the release of buried seeds from dormancy occurs as the range of environmental conditions which are suitable for germination is widened. It apears to be a general rule that germination in the field occurs when conditions in the habitat coincide with this range (Vegis 1964). The germination responses to temperature regime recorded on the thermogradient bars are only relevant to the field situation at a particular date where the bar temperatures overlap with field temperatures. For example, a fluctuation of 7-28°C would never be experienced in the field in winter.

Temperature data were obtained from Plymouth Polytechnic Experimental Station, Rumleigh, in 1981, by placing thermocouples just below the soil surface. The resulting graphs of daily maximum and minimum temperatures and temperature range or amplitude of fluctuations (Fig. 4.9) show that the minimum temperatures did not rise above 7°C before July and the maximum temperatures were generally between 10-20°C until the first week in June when there was a distinct rise in maximum temperature values. The amplitude of temperature fluctuations generally fell between 5-16°C until mid-June, after which amplitudes of 20-25°C were occasionally recorded. If these temperatures are compared with those experienced by soil on the thermogradient bars (Fig.4.1) it can be seen that field temperatures frequently overlap with temperatures in sections 2 and 3 between April and July but temperatures in sections 4 and 5 only occur in the field in June and July. Section 1 temperatures do not overlap with those experienced in the field.

Rainfall was also measured at this site and is shown in Fig. 4.9. Emergence of seedlings from the naturally buried seed population was recorded at approximately ten day intervals in nine permanent quadrats (20cm x 20cm) at this site. The soil was bare and spaces between the quadrats were weeded regularly. It was hoped to correlate flushes of emergence of the main weed species with changes in absolute temperatures, temperature fluctuations and rainfall and to compare this with what might be expected from the results of experiments on the thermogradient bars.

FIG. 4.9.

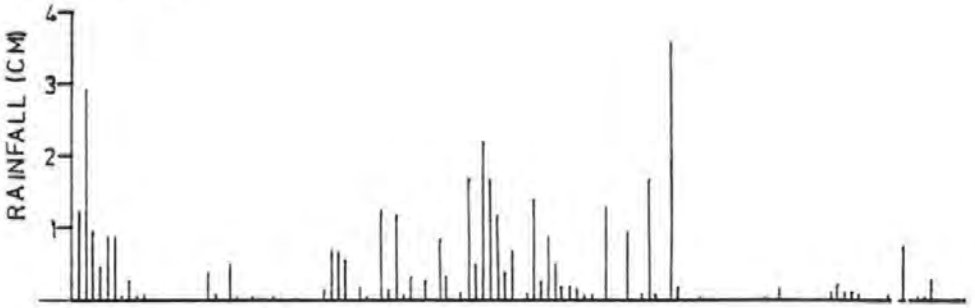
Rainfall and temperature data (1981) from Plymouth Polytechnic Experimental Station, Devon.

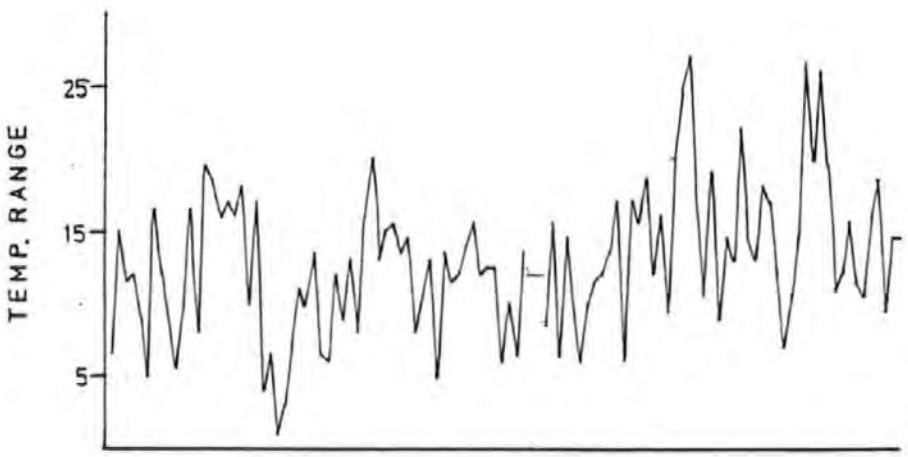
Daily maximum and minimum temperatures were recorded just below the bare soil surface and the temperature range or amplitude of fluctuations calculated from the data.

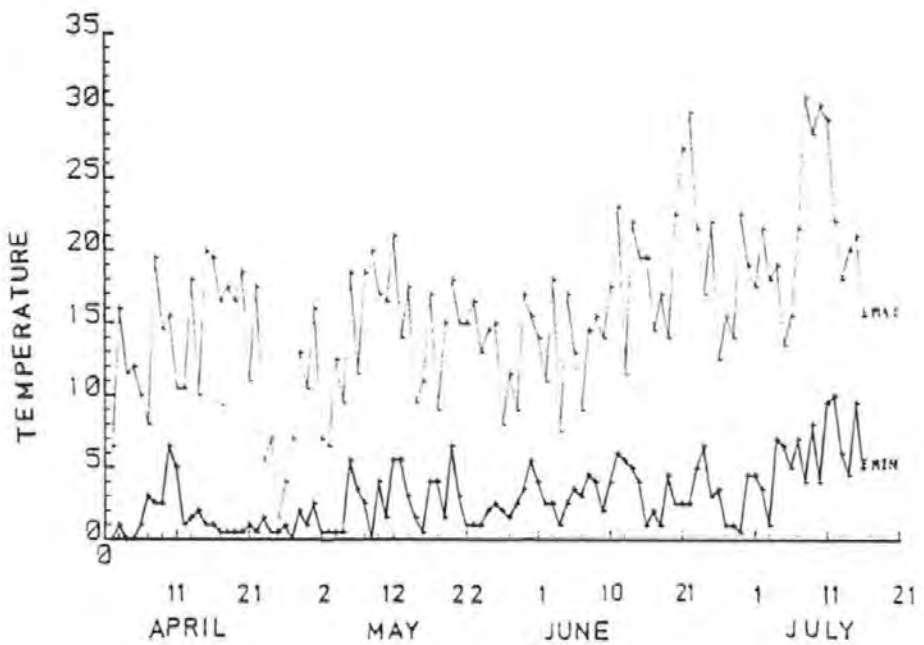
The acetate overlay shows the mean total number of seedlings recorded in nine permanent quadrats at approximately 10 day intervals at this site.

KEY Epilobium tetragonum —— Coronopus didymus ·····

FIG. 4.9


RAINFALL (CM)
4
3
2
1


TEMP. RANGE
25
15
5


TEMPERATURE
35
30
25
20
15
10
5
0
0
TMAX
TMIN
11
21
2
12
22
1
10
21
1
11
21
APRIL
MAY
JUNE
JULY

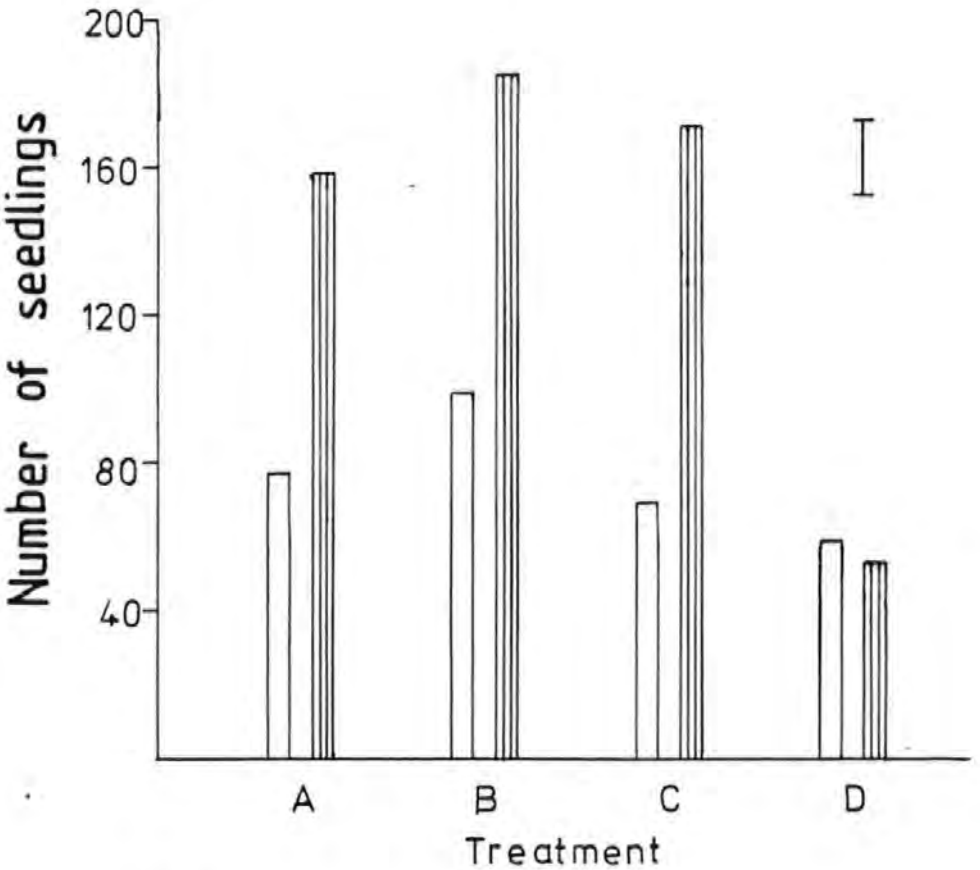


FIG. 4.10

KEY

□ Natural moisture conditions.

▥ Watered regularly.

The effect of various soil surface treatments on the mean total number of seedlings emerging in each of five 20 x 20 cm quadrats during the period 12-5-82 to 16-6-82.

Treatments :- bare soil (A); covered with a layer of green netting (2mm. mesh size) (B); covered with a thin layer of perlite (3-4 mm.) and netting (C); covered with a thick layer of perlite (12 mm.) and netting (D). Bar indicates L.S.D. (P = 0.05).

The feld emergence of two common weed species tested on the bars, Epilobium tetragonum (Fig.4.5) and Coronpus didymus (Fig.4.3) did not appear to correlate with temperature changes (Fig. 4.9). This was not surprising as the results for these species from the thermogradient bars suggest that the temperature fluctuations experienced in the field were nearly always big enough to permit maximum germination during the period shown in Fig.4.9. The marked drop in seedlings from the beginning of June onwards was probably related to lack of soil moisture.

Influence of soil moisture on seedling emergence

Evidence was collected in support of the importance of the influence of soil moisture on seedling emergence from site 2 (the derelict pasture). Gaps were cut in the vegetation and wire quadrats were fixed on the bare soil surface (Treatment A), covered with a layer of green netting (Treatment B), with a thin layer of perlite and netting (Treatment C) or a thick layer of perlite (12mm) and netting (Treatment D). Each treatment included six replicates, three of which were watered regularly and the rest remained under natural moisture conditions. Seedling emergence was recorded at weekly intervals between 12-5-82 and 16-6-82. The mean total number in each replicate for the different treatments over this period is shown in Fig. 4.10. The analysis of variance test showed significant differences (P=0.05) between the seedling numbers in wet and dry quadrats in treatments A,B and C but not D. The thick layer of perlite granules prevented evaporation from the soil surface and the natural rainfall received was conserved. This effect may only be important on bare soil at cultivated sites or in large gaps in pastures such as this when moisture can evaporate rapidly in the summer. However, soil samples

collected from sites 2,3 and 4 on 23-6-83 after several weeks of dry weather show that the soil had quite a low moisutre content even when collected from beneath the intact canopy at site 2 (Table 4.1).

TABLE 4.1

Percentage soil moisture at two depths on 23-6-83.

	DEPTH	
	SOIL SURFACE	5cm BELOW
Site 2 (Derelict pasture)	16.1	13.6
Site 3 (Daffodil field)	3.2	13.6
Site 4 (Potato field)	3.3	11.8

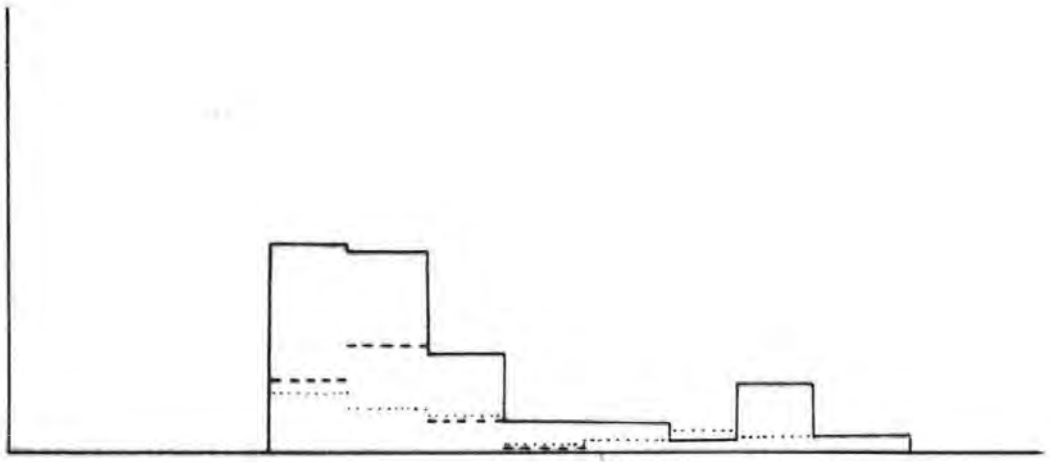
The vegetation at site 2 did reduce the amount of evaporation from the soil surface compared with sites 3 and 4, but a moisture content of 16.1% is still likely to affect seed germination. Benjamin (1974) concluded from controlled experiments on the effect of moisture on seed germination of three weed species, that some germination is possible at any soil moisture content of 10% or above and that above 10% was linearly related to moisture content, at least up to 20% moisture content.

Because of the important effect of soil moisture on seedling emergence patterns an experiment was designed to eliminate its effects by keeping the soil at field capacity throughout the period of observation.

Seedling emergence from soil kept at field capacity

This experiment was carried out in 1983 at Plymouth Polytechnic and is described in detail in Chapter 5 but some of the seedling emergence data which can be compared with the thermogradient bar tests discussed here is illustrated in Fig.4.11. Temperatures were measured just below the soil surface and the soil was exposed to light. The range of temperature fluctuations was similar to that recorded in 1981 for the corresponding months. The amplitude of temperature fluctuations in January and February generally fell between 1.5 and 6.0°C and is similar to the temperature regime in section 1 of the thermogradient bars (Fig.4.1).

Results from the thermogradient bars suggest that Coronpus didymus (Fig.4.3) would not be affected by temperature fluctuations in early spring but that the optimum amplitude of temperature fluctuations for emegence of Epilobium tetragonum (Fig.4.5) may gradually increase from approximately 8 -16°C in January to



KEY Epilobium tetragonum —

Coronopus didymus .....

Polygonum aviculare ----

approximately 13 -20°C by mid-May. The seedling emergence figures for Epilobium tetragonum (Fig. 4.5) also suggest a general drop in numbers at all temperatures by mid-May. The number of Polygonum aviculare seedlings emerging on the thermogradient bars (Fig.4.6) was very low in May and the optimum temperature fluctuation range was fixed at approximately 20°C in both January and May.

When the field emergence pattern is studied there seems to be a significant chilling effect due to the cold period in February as there is a large flush of seedlings of all three species as soon as the minimum temperatures rise above 0°C. This effect overcame the lack of stimuation by small temperature fluctuations which was predicted from tests on the thermogradient bars. It is well known that the dormancy of Polygonum aviculare is broken by chilling (Courtney 1968) but the chilling effect is unexpected in Epilobium tetragonum and Coronopus didymus. There was no effect of chilling on harvested seeds of four other species of Epilobium (Grime et. al. 1981) suggesting that burial affected the germination response of these species in some way.

Apart from the chilling effect, the results conform very well with the thermogradient bar results. The relatively greater germination of Epilobium tetragonum and Coronopus didymus compared with Polygonum aviculare is predicted at low temperature fluctuations and the increased germination of Polygonum aviculare after exposure to larger temperature fluctuations of approximately 15°C is also predicted.

FIG. 4.11.

Temperatures recorded just below the surface of a layer of bare soil on the roof of Plymouth Polytechnic. (see Plate 4, back of thesis).

Acetate overlay shows the total numbers of seedlings that emerged from naturally buried seeds in the soil sample.

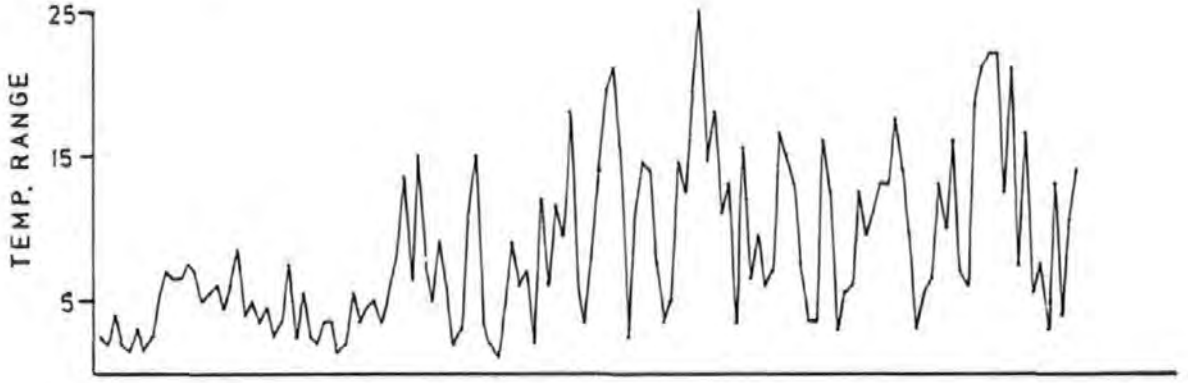
Seedlings were recorded and removed at approximately ten day intervals.

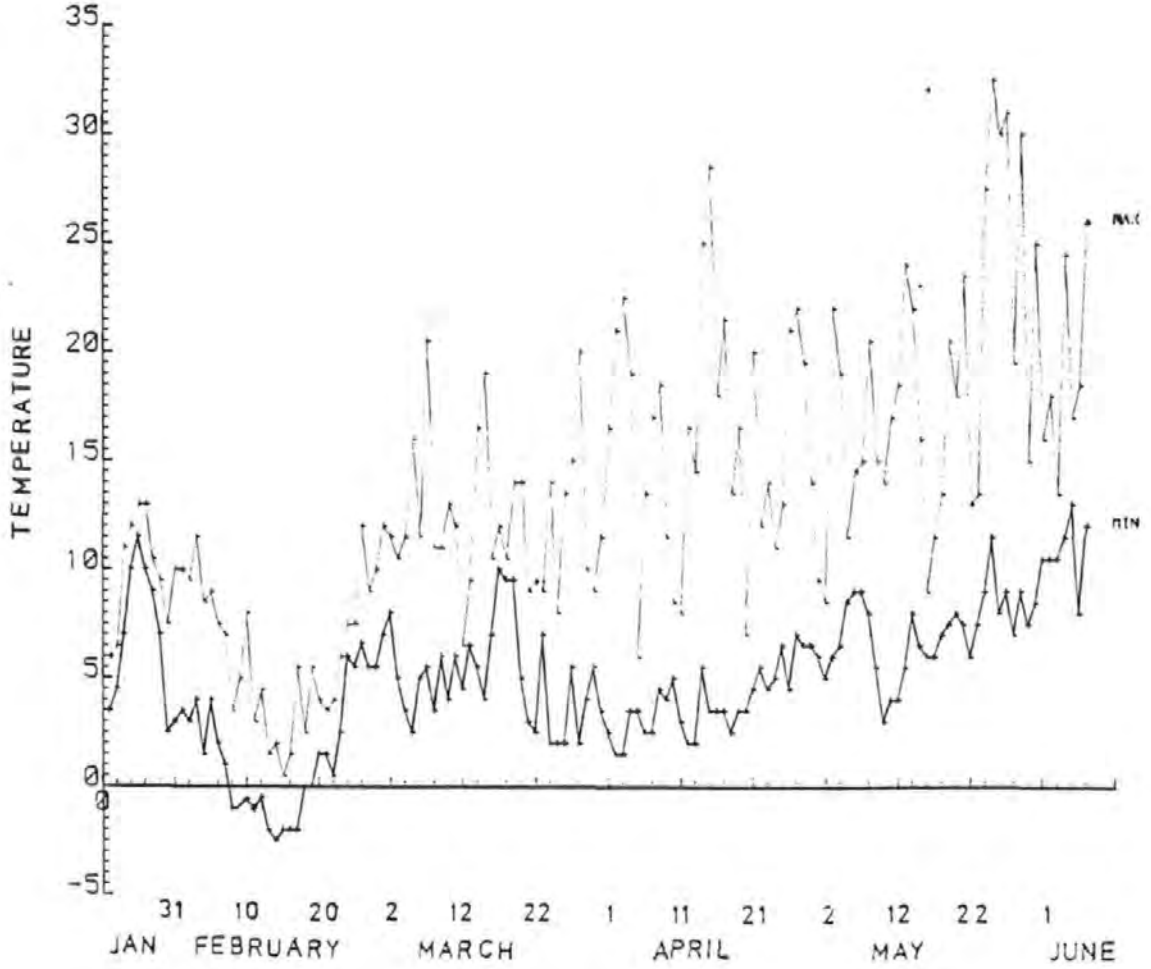
SUMMARY

Significant seasonal changes in the response of buried seeds to a fluctuating temperature regime were only found in two out of the six species tested. It has already been suggested that the changes found in Coronopus didymus were caused by a large influx of freshly shed seeds into the soil on a particular sampling date and were thus more apparent than real. There were generally distinct differences in response between species, which emphasises the need for screening experiments on a wide range of species with persistent buried seed reserves.

The results from the thermogradient bars accurately predicted the seedling emergence pattern when there was a distinct change in the amplitude of temperature fluctuations during the period studied (Fig.4.11). In a time of generally large fluctuations in a homogenous, open habitat (Fig.4.9) one might expect that seedling emergence would be governed by factors other than temperature fluctuations, such as moisture availability or nitrate levels (Benjamin 1974). In a heterogenous environment, with patches of open and closed vegetation cover, responses to temperature fluctuations may be important in determining the location rather more than the timing of germination. This possibility was investigated in more detail in an experiment described in the folowing chapter.

FIG. 4.11


TEMP. RANGE
25
15
5


TEMPERATURE
35
30
25
20
15
10
5
0
-5
31
10
20
2
12
22
1
11
21
2
12
22
1
JAN
FEBRUARY
MARCH
APRIL
MAY
JUNE

CHAPTER 5

GERMINATION RESPONSES OF NATURALLY BURIED SEEDS TO FLUCTUATING TEMPERATURES

Introduction

The stimulatory effect of diurnal temperature fluctuations on germination is known to be widespread (Thompson, Grime and Mason, 1977). Much work has been done using harvested seeds to investigate their response to fluctuating temperatures (Totterdell and Roberts 1980, Thompson and Grime 1983) and the

positive interactions found between fluctuating temperatures and other stimulatory factors such as light and nitrate (Vincent and Roberts, 1977 and Roberts and Benjamin, 1979).

For reasons described in Chapter 3, naturally buried seeds are likely to respond differently from harvested seeds to dormancy breaking stimuli, particularly as a light requirement can develop during burial (Wesson and Wareing, 1969 b , Taylorson, 1970). The purpose of this investigation was, therefore, to study the responses of naturally buried seeds to temperature fluctuations in the light and dark.

There are a number of attributes of alternating temperature regimes which could conceivably affect seed germination. These include the number of cycles, the amplitude of temperature difference, the value of the upper and lower temperatures, the time spent at the upper and lower temperature within each cycle, the rate of warming and cooling and the timing of the cycles with respect to the start of imbibition (Totterdell and Roberts, 1980). In the following experiments only the effect of the amplitude of temperature differences and values of the upper and lower temperature in each cycle were studied. The other attributes were kept constant.

Species tested on the thermogradient bars

The species used on the bars were selected according to the germination requirements shown by their harvested seeds (Thompson 1977, and Thompson and Grime 1983). They included species which required temperature fluctuations for germination in the light (e.g.Juncus species), those in which the response to temperature fluctuations was greater in the dark than in the light (e.g.Stellaria media), those in which the response to temperature fluctuations occurred only in continuous darkness (e.g.Holcus lanatus) and those which showed very little response to temperature fluctuations in the light or darkness (e.g. Poa annua).

Some of the sites at which buried seeds of several species were collected are described at the beginning of Chapter 4 and illustrated in Plates 4.1, 4.2 and 4.3. Two further sites were selected at Warleigh Point Nature Reserve (Nat. Grid Ref. SX 449 608). One was a marshy area with soil containing large numbers of Juncus acutiflorus while the other was a recently coppiced area of woodland with largely bare ground covered by patches of Rubus species. The soil at this second site contained large numbers of seeds of Digitalis pupurea and Hypericum perforatum. Finally, soil containg buried seeds of Plantago major and Agrostis stolonifera was collected from a grass verge at Plymouth Polytechnic Experimental Station.

Fourteen species of buried seeds were tested on the thermogradient bars in the light and the dark and five of these species were also investigated under somewhat controlled field conditions where the patterns of seedling emergence were recorded. The seed environment was modified in this field experiment to reduce the mean amplitude of temperature fluctuations experienced by the seeds in one treatment by insulating the soil surface. It was hoped

to verify predictions made from tests on the thermogradient bars by correlating seedling emergence with temperature fluctuations in the field.

Germination tests on the thermogradient bars

The soil was collected from the field and prepared only a few days before it was tested on the thermogradient bars to avoid changes in the dormancy state of seeds due to dry storage. The soil sampling and preparation technique is described in Chapter 2.

The temperature regime used on the thermogradient bars is illustrated in Fig.5.1. The amplitude of the temperature fluctuations varied between 1.5 and 25°C and the temperature at the 'constant' end of the bars was maintained at approximately 12°C. At the opposite end of the bars the maximum and minimum temperatures experienced by seeds in the soil were 31°C and 6°C. The upper temperature was given for 10 hours and the lower temperature for 14 hours, including a dark period of 10 hours. Soil to be tested in darkness was spread on the lower bar (see Plate 2) and was covered by a layer of sterile sand (2-3mm) which prevented light from reaching the seeds in the soil. These temperature and light regimes are similar to those experienced in the field in surface layers of soil in late spring.

The layer of dry, prepared soil (5-7mm thick) on each bar was re-wet to field capacity using a fine mist sprayer and the apparatus sealed to conserve moisture (further details in Chapter 2). Each batch of soil tested on the thermogradient bars was subjected to fourteen cycles of temperature fluctuations (i.e. 14 days) before the number of seedlings was recorded.

In addition to the experiments that used a fluctuating temperature regime, soil from site 2 (i.e. that containing Holcus

lanatus and Rumex obtusifolius) was tested in the light using each of the 'day' and 'night' gradients shown in Fig. 5.1. That is, the bar was operated as a normal thermogradient bar with a constant gradient rather than with a fluctuating gradient. This experiment was designed to reveal the germination responses of buried seeds of Holcus lanatus and Rumex obtusifolius to constant temperatures over the range of 6°C to 31°C. Soil from the same collection was again tested at the fluctuating regime for comparative purposes. It was hoped that the results would indicate whether germiation responses on the bars were caused by fluctuations per se or by maximum and minimum temperatures encompassed by the fluctuations.

Data collection and analysis

In order to express the distribution of seedling emergence graphically the six chambers formed by the wooden partitions were divided into five sections, each with the same increment in the number of degrees of temperature fluctuation experienced. The area of each section was calculated using the graph shown in Fig. 5.1 and each section was assigned a mean amplitude of temperature fluctuation. Since the sections were of unequal area, the numbers of seedlings were expressed on a per unit area basis.

Valid statistical comparisons could only be made using data from soil samples collected at a particular site on the same sampling date. To investigate the effect of differences in the mean amplitude of temperature fluctuations in each section, while taking account of random variation between the replicate treatments, the analysis of variance technique was used. A value for the L.S.D. between mean seedling numbers in each soil test was calculated from the relevant analysis of variance table and these are indicated on Figs. 5.2-5.8.

FIG. 5.1.

Temperature gradients at the bar surface during operation of the regime used in current experiments. Gradients maintained for ten hours (O) and fourteen hours (●) in every 24. A ten hour dark period was included in the latter period.

FIGS. 5.2 - 5.8.

Numbers of seedlings emerging on the thermogradient bars in response to different amplitudes of temperature fluctuations.

Soil containing naturally buried seeds was tested in a fourteen hour photo period (O) and in the dark (●). Bars indicate L.S.D. (P = 0.05).

FIG. 5.1.

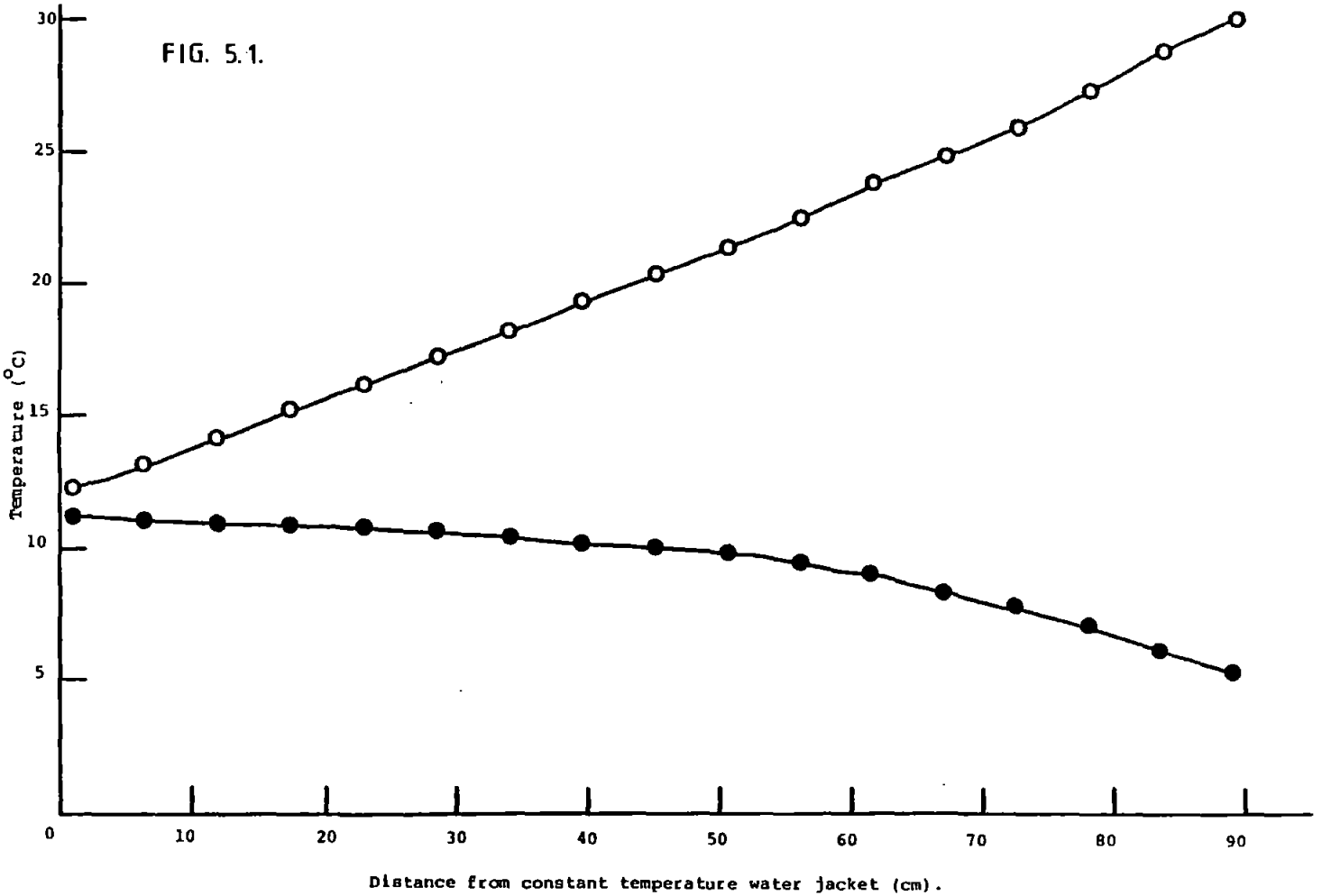

30
25
20
15
10
5
0
Temperature (°C)
10
20
30
40
50
60
70
80
90
Distance from constant temperature water jacket (cm).

FIG. 5.2

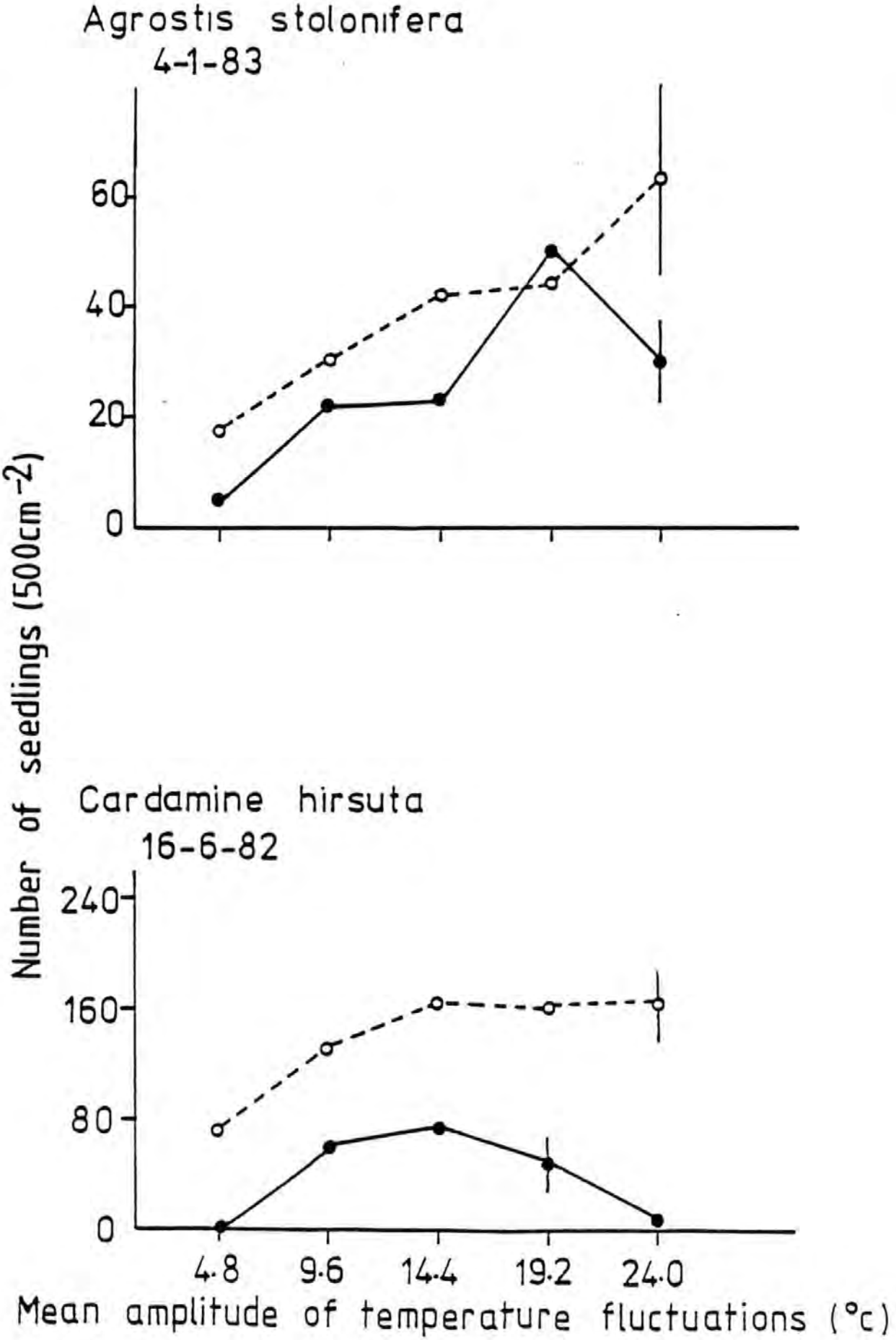

Agrostis stolonifera
4-1-83
60
40
20
0
Cardamine hirsuta
16-6-82
240
160
80
0
Number of seedlings (500cm⁻²)
4·8
9·6
14·4
19·2
24·0
Mean amplitude of temperature fluctuations (°c)

FIG. 5.3

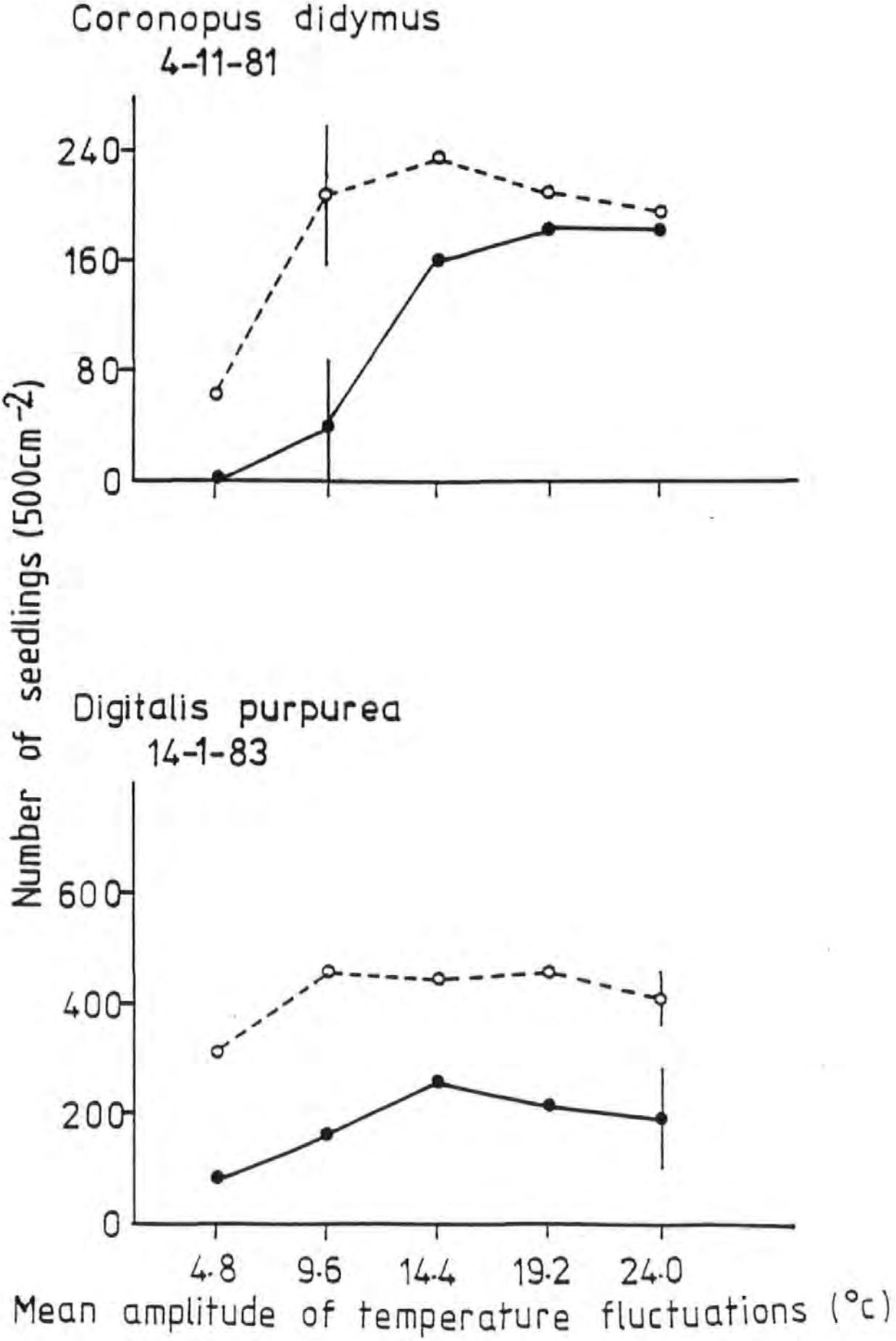

Coronopus didymus
4-11-81
240
160
80
0
Digitalis purpurea
14-1-83
600
400
200
0
Number of seedlings (500cm⁻²)
4·8
9·6
14·4
19·2
24·0
Mean amplitude of temperature fluctuations (°c)

FIG. 5.4

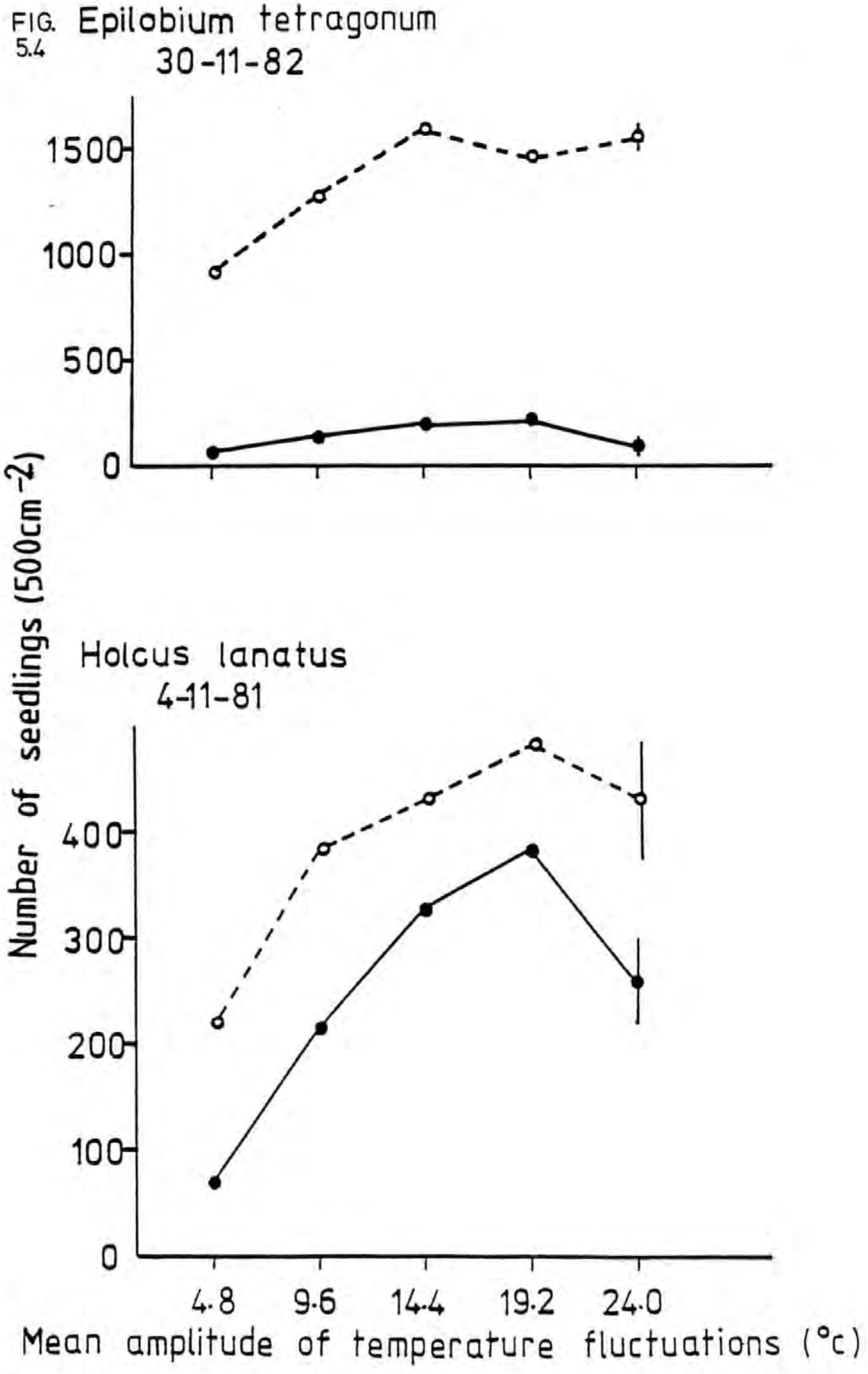

Epilobium tetragonum
30-11-82
1500
1000
500
0
Holcus lanatus
4-11-81
400
300
200
100
0
Number of seedlings (500cm⁻²)
4·8
9·6
14·4
19·2
24·0
Mean amplitude of temperature fluctuations (°c)

FIG. 5.5

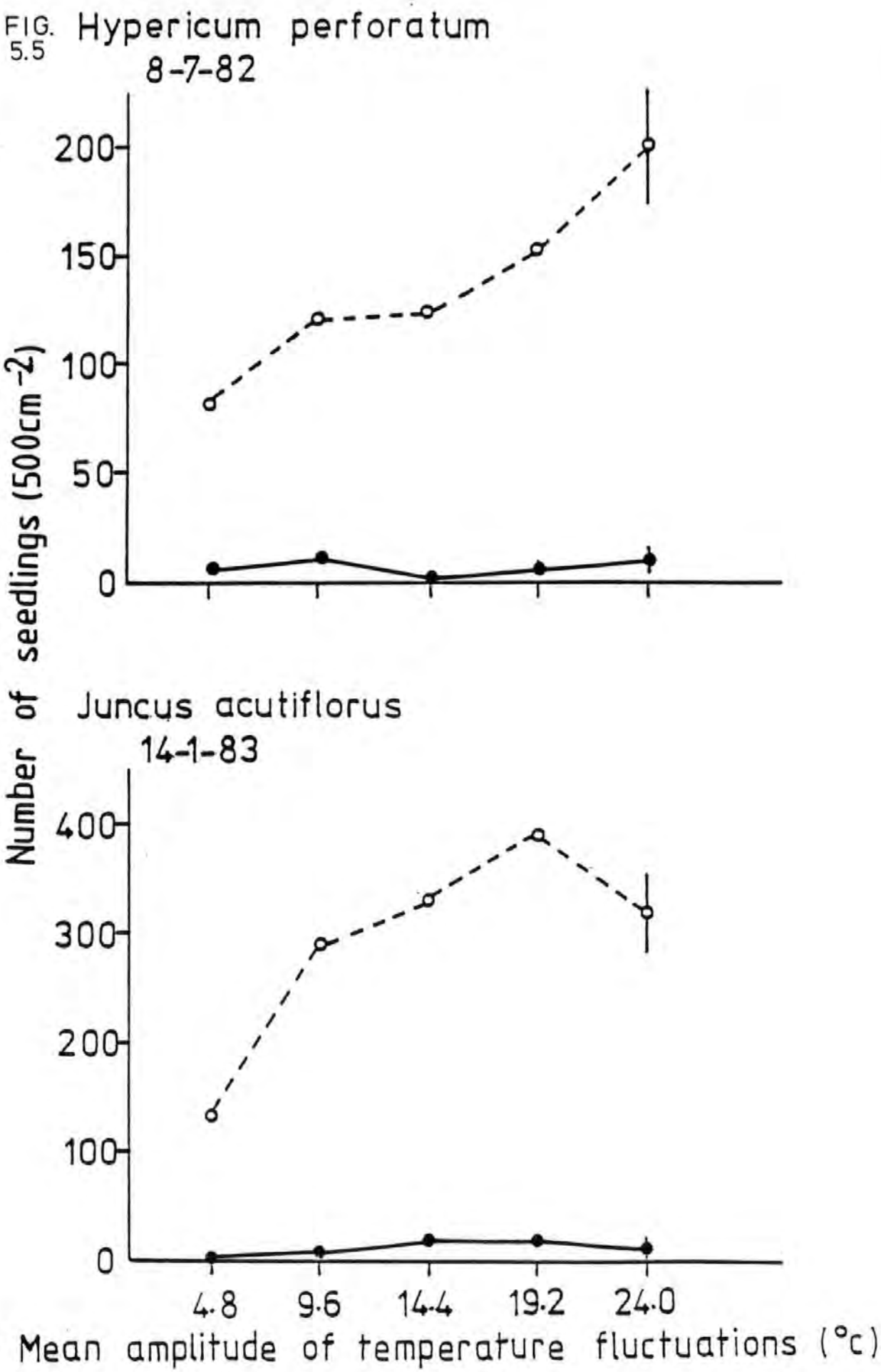

Hypericum perforatum
8-7-82
200
150
100
50
0
Number of seedlings (500cm⁻²)
Juncus acutiflorus
14-1-83
400
300
200
100
0
4.8
9.6
14.4
19.2
24.0
Mean amplitude of temperature fluctuations (°c)

FIG. 5.6

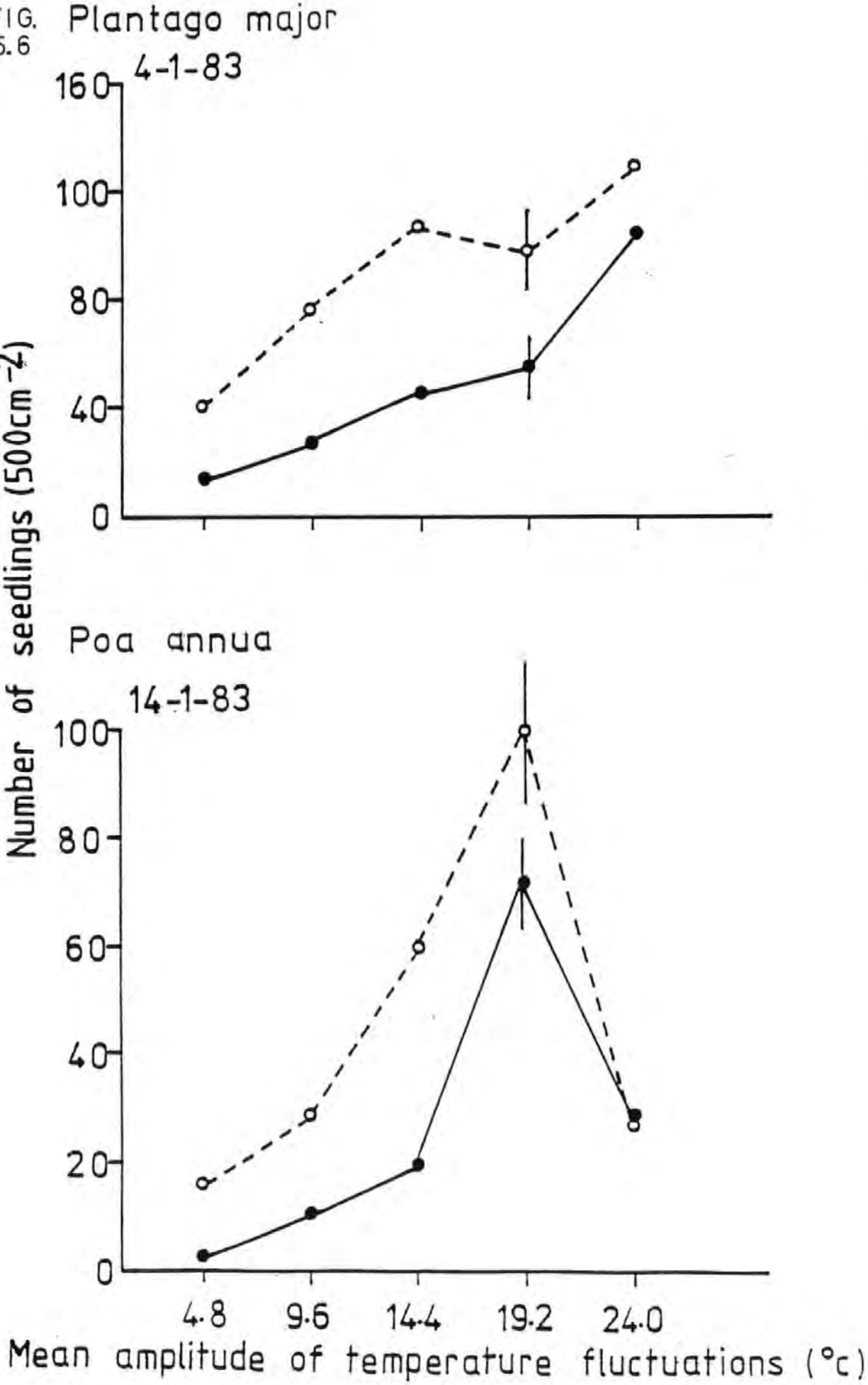

Plantago major
4-1-83
160
100
80
40
0
Poa annua
14-1-83
100
80
60
40
20
0
Number of seedlings (500cm⁻²)
4·8
9·6
14·4
19·2
24·0
Mean amplitude of temperature fluctuations (°c)

FIG. 5.7

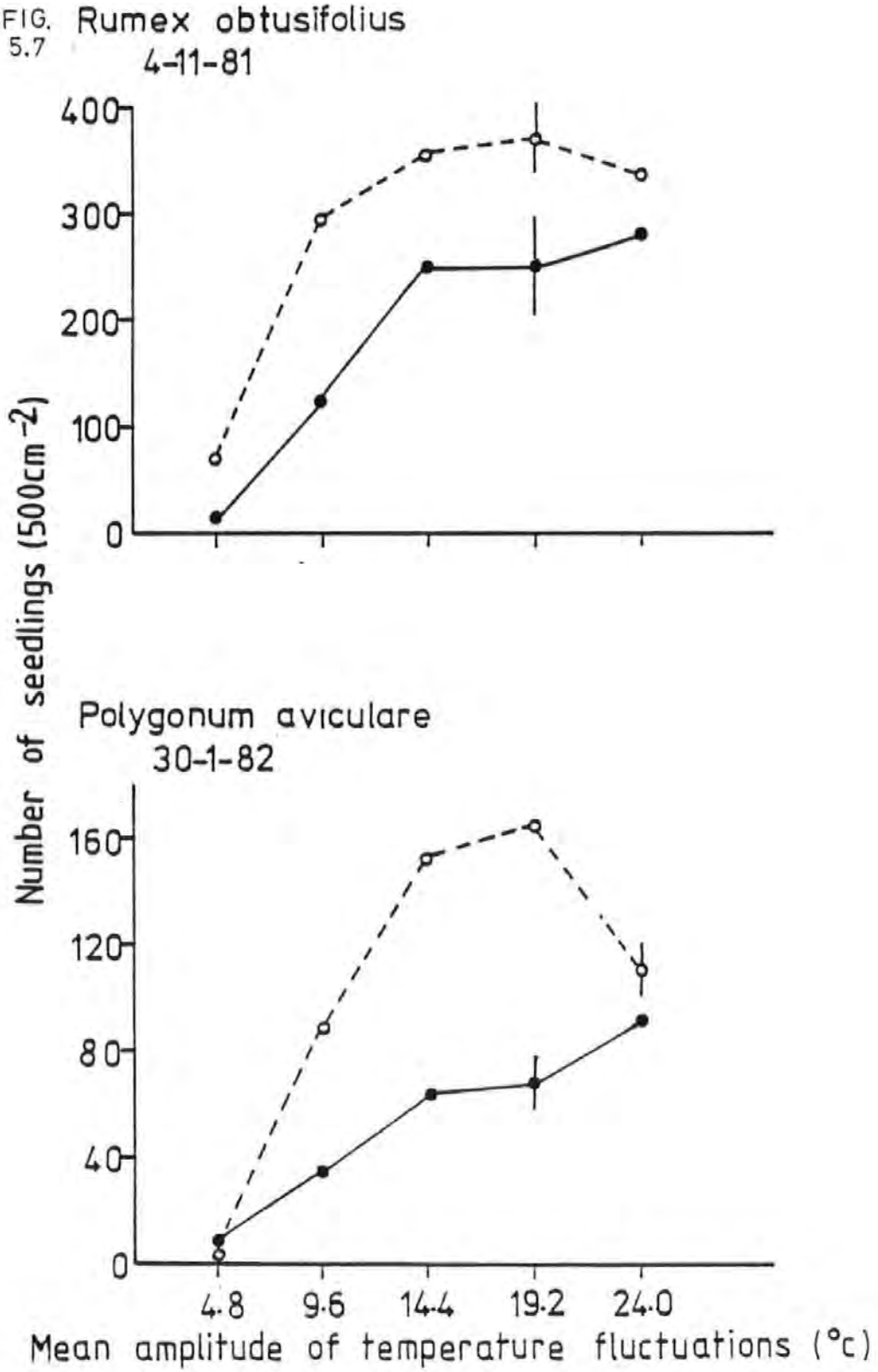
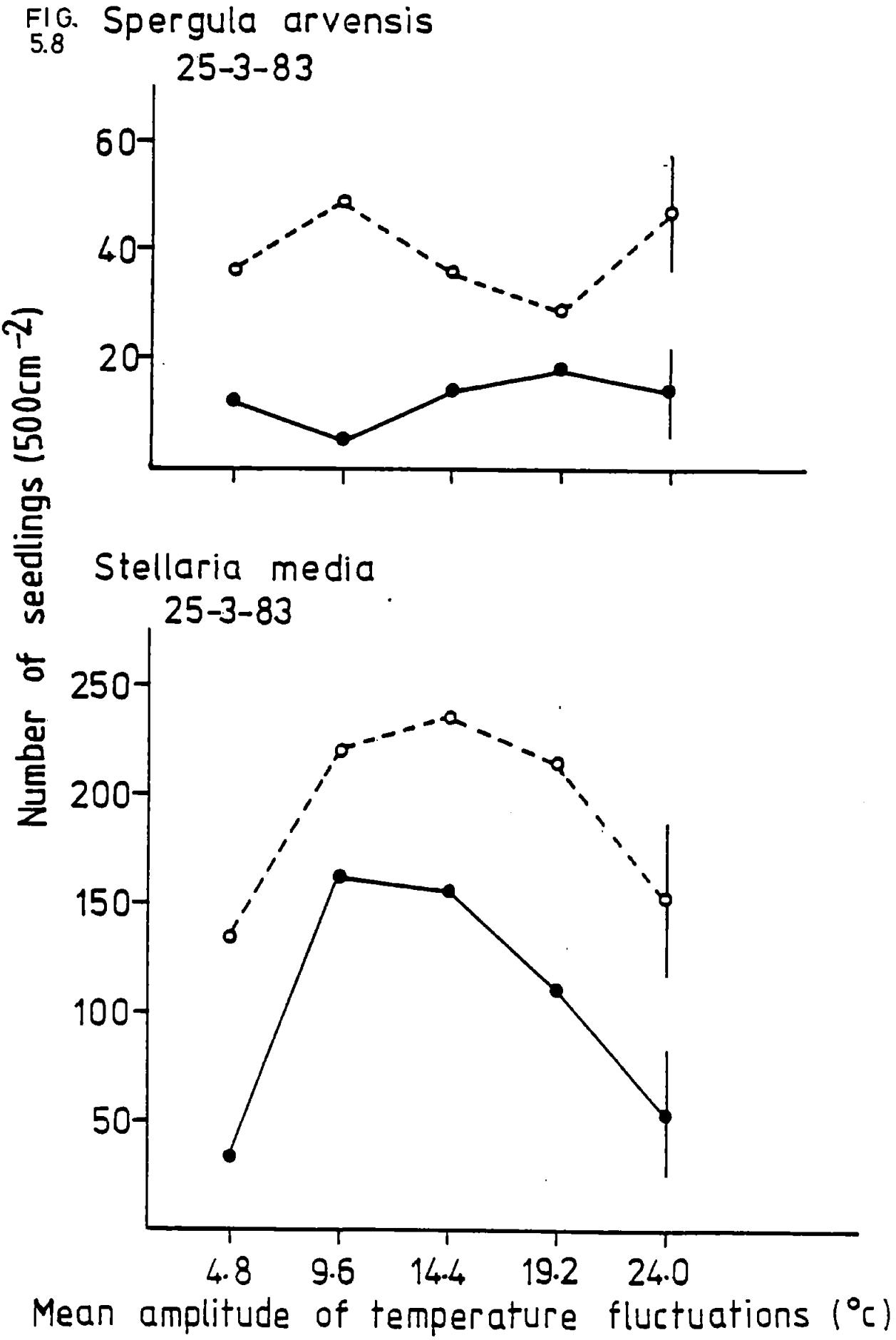

Rumex obtusifolius
4-11-81
400
300
200
100
0
Number of seedlings (500cm⁻²)
Polygonum aviculare
30-1-82
160
120
80
40
0
4·8
9·6
14·4
19·2
24·0
Mean amplitude of temperature fluctuations (°c)

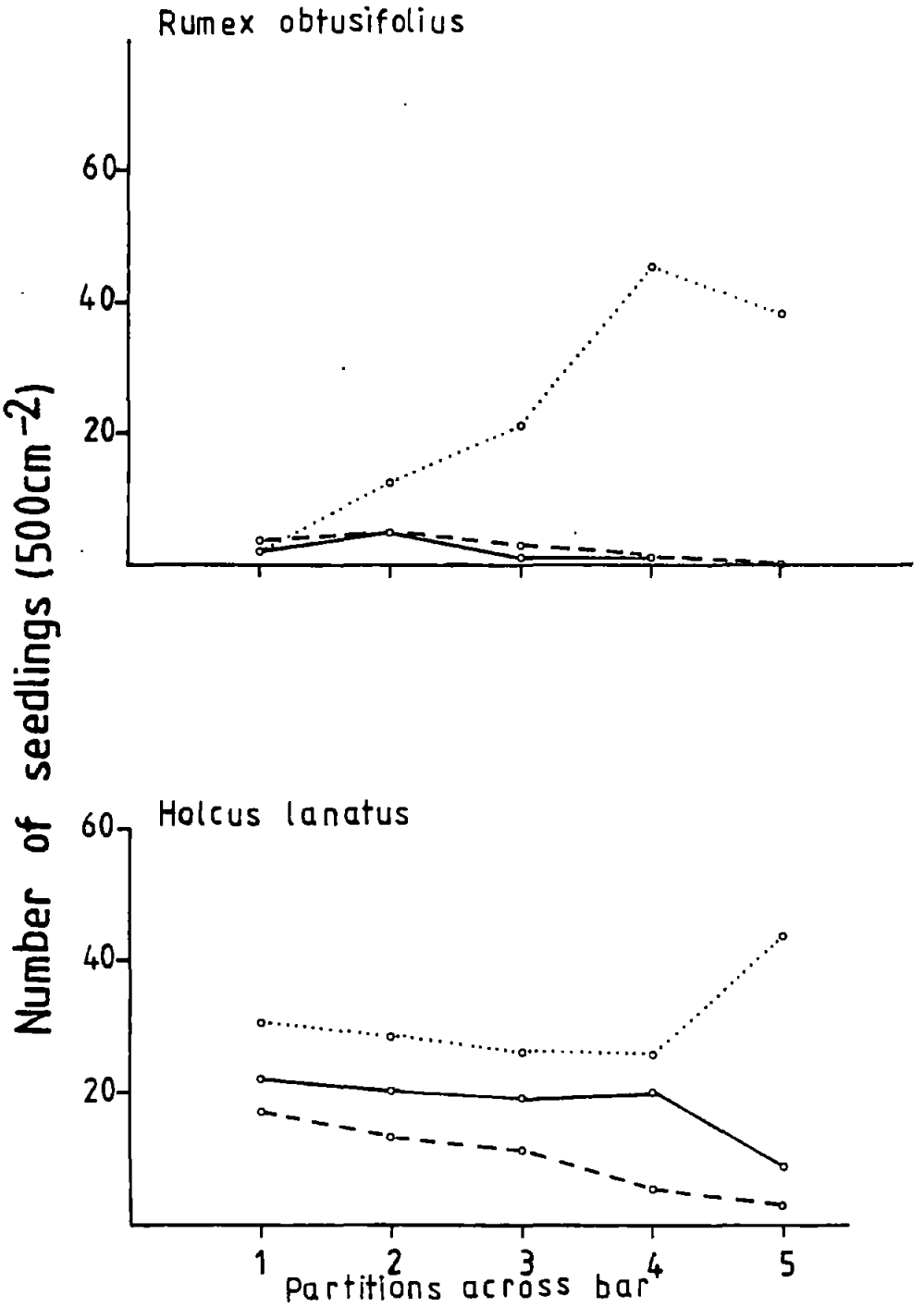
FIG. 5.8


Spergula arvensis
25-3-83
60
40
20
Stellaria media
25-3-83
250
200
150
100
50
Number of seedlings (500cm⁻²)
4·8
9·6
14·4
19·2
24·0
Mean amplitude of temperature fluctuations (°c)

5.9

Response of naturally buried seeds to the 'daytime' gradient of constant temperatures (12 - 30°c) o------o; to the "night-time" gradient of constant temperatures (11 - 6°c) o———o; and to the normal fluctuating temperature regime (see Fig. 5.1) o·····o.

FIG. 5.9


Rumex obtusifolius
60
40
20
Holcus lanatus
60
40
20
Number of seedlings (500cm⁻²)
1
2
3
4
5
Partitions across bar

RESULTS

Responses to temperature fluctuations in the light

The tests on the light bar simulated field conditions in which seeds that had been buried for some time were exposed to light, for example by ploughing or by the burrowinng activities of moles. In the field situation the degree of exposure and slope of each microsite would determine the amplitude of temperature fluctuation experienced by individual seeds.

Stimulation of germination by temperature fluctuations in the light was found in at least twelve of the fourteen species examined. In the majority of species the effect of large fluctuations was merely to increase the number of germinations found at the lowest fluctuation (4.8°C) but in one, Polygonum aviculare (Fig. 5.7), there was an obligate requirement for fluctuations in excess of this value. Several species required large fluctuations (e.g. >9°C in Agrostis stolonifera (Fig.5.2) and Poa annua (Fig.5.6) to achieve 50% of the maximum number of seedlings recorded. Conversely, in other species (e.g. Epilobium tetragonum (Fig.5.4) and Stellaria media (Fig.5.8)) 50% of the maximum number of seedlings was attained at fluctuations of approximately 5°C.

Over half of the species examined showed either no further increase or a marked decrease in the number of seedlings emerging when the mean amplitude of temperature fluctuations exceeded approximately 19°C, (e.g. Juncus acutiflorus (Fig.5.5), Polygonum aviculare and Poa annua). The heterogeneity of behaviour found for single species within a population of seeds, which has already been demonstrated for harvested seeds (Thompson and Grime 1983 and personal observations described in Chapter 3 of this thesis), was again evident. In most species this took the form of a smooth increase in the number of seeds

germinating as the amplitude of temperature fluctuations increased. In a minority of species there was a more obvious 'step' in germination between mean amplitudes of 4.8°C and 9.6°C (e.g. Rumex obtusifolius (Fig.5.7) and Coronopus didymus (Fig. 5.3). There was a suggestion in some species (e.g. Hypericum perforatum (Fig.5.5), Plantago major (Fig.5.6) and Agrostis stolonifera) that a further increase in the amplitude of fluctuation above 24°C, the maximum employed here, would have resulted in a further increase in the number of seedlings emerging.

Responses to temperature fluctuations in darkness

The soil tests on the dark bar simulated field conditions in which the seeds remained buried. In the field different amplitudes of temperature fluctuations would be caused by differences in the depth of seed burial and differences in the insulating effect of a litter layer or leaf canopy.

It can be seen from the figures that, with few exceptions, the effect of darkness was to reduce the number of seedlings emerging from a soil sample, even at large temperature fluctuations. Some species (e.g.Hyperium perforatum and Juncus acutiflorus) showed almost no germination in the dark, irrespective of temperature regime. In several species (e.g. Epilobium tetragonum, Poa annua) the response to increasing amplitudes of temperature fluctuations was more gradual in the dark than in the light.

In many species the shape of the response curve to increasing temperature fluctuations was different in the dark from in the light, the main difference being a narrower optimum temperature range for germination in the dark. The drop in seedling numbers emerging when the amplitude of temperature fluctuations exceeded 19°C was generally

more marked in the dark than in the light (e.g. Cardamine hirsuta, Fig.5.2), Agrostis stolonifera and Holcus lanatus, Fig.5.4)), but Polygonum aviculare and Rumex obtusifolius were exceptional in this respect.

Response of Holcus lanatus and Rumex obtusifolius to constant temperatures

The results (Fig.5.9) show that germination was more stimulated by fluctuating temperatures than by constant temperatures over the whole range for both Rumex obtusifolius and Holcus lanatus. Very few seeds of either species germinated when exposed to constant temperatures corresponding to the extremes covered by the large temperature fluctuations which produced maximum germination.

DISCUSSION

The interaction between amplitude of fluctuation and absolute temperature

In studies of the effects of fluctuating temperatures it is often difficult to distinguish between responses to fluctuations per se and to the absolute temperatures experienced during these fluctuations. This may be a problem in the present study, where there is a tendency for germination to decline at large amplitudes of fluctuation, which necessarily encompass both rather low and rather high absolute temperatures. The results obtained here can be compared with the upper and lower constant temperature limits for 50% of maximum germination in the light, as established by Grime et.al. (1981). The data for harvested seeds of some of the species tested on the thermogradient bars are shown in Table 5.1.

TABLE 5.1

The maximum and minimum constant temperatures at which germination attained 50% of maximum (tested in the light). Seeds were stored dry at 5°c before use. Data from Grime et. al. (1981).

SPECIES	MINIMUM TEMP. (°C)	MAXIMUM TEMP. (°C)
Agrostis stolonifera	11	36
Cardamine hirsuta	11	28
Digitalis purpurea	10	29
Holcus lanatus	<5	35
Hypericum perforatum	13	35
Plantago major	15	34
Poa annua	7	31
Rumex obtusifolius	15	28
Spergula arvensis	<5	35
Stellaria media	10	28

Unfortunately only a minority of species show a reduction in germination at high fluctuations in the light of the present study, and not all of these are included in Grime's paper, so it is difficult to draw any general conclusions. In both Holcus lanatus and Poa annua there is a decline in germination at high fluctuations (although this is only slight in Holcus lanatus), yet the upper and lower limits for germination at constant temperatures are very wide, suggesting the reduction is an effect of fluctuations per se. In Stellaria media and Rumex obtusifolius the inhibition of germination at large fluctuations does correspond to exposure to temperatures (both high and low) which are outside the limits for high germination at constant temperatures. In contrast in at least one species (Cardamine hirsuta) absolute temperatures both above and below those conducive to high germination at constant temperatures are not associated with a reduction in germination when these temperatures are experienced as part of a fluctuating regime.

In those species (e.g. Hypericum perforatum and Agrostis stolonifera) where germination at constant temperatures extends to very high temperatures (>35°C) but not to low temperatures, it seems clear that fluctuating temperatures can extend the lower end of the germination temperature range. This effect is already well known from studies of the effects of fluctuating temperatures on harvested seed (Thompson and Grime 1983).

Despite what has been said above, there are great difficulties in comparing the data in Figs.5.2 to 5.8 with Table 5.1. Sources of variation between the two sets of data include differences in behaviour between harvested and buried seeds, and possible ecotypic differences as well. Fortunately both these problems are overcome for Holcus lanatus and Rumex obtusifolius, for which data are available

concerning the response of the same population of buried seeds to both constant and fluctuating temperatures (Fig.5.9). The results in Fig.5.9 suggest that quite strong inhibition by certain constant temperatures is overcome by the inclusion of these temperatures in a fluctuating regime. This is particularly clear in Rumex obtusifolius, where high germination is obtained from a fluctuating temperature regime even when both the constant temperatures alone are almost completely inhibitory. There is again a suggestion, however, that at the largest fluctuation germination is reduced, i.e. the effect of the fluctuation is not quite sufficient to counter the effect of the two highly inhibitory constant temperatures.

Discussion of the Holcus lanatus results is slightly complicated by the fact that the response to fluctuating temperatures is rather different than that previously obtained (Fig.5.4) from the same population. Nevertheless, it is still clear that even though extreme constant temperatures partly inhibit germination in this species, the combination of these temperatures in a fluctuating cycle is highly stimulatory to germination. Unlike Rumex obtusifolius, where small (4.8°C) fluctuations have little effect, germination of Holcus lanatus is increased by fluctuating temperatures of all amplitudes above the level obtained by the constant temperatures separately.

Constant temperature limits for germination in the dark are not known for buried or harvested seeds and therefore little can be said about the interaction between absolute and fluctuating temperatures in the dark. It seems reasonable to assume, however, that the general principles established above will apply in the dark, with the reservation that both constant and fluctuating temperature limits will be very much narrower than in the light (Thompson and Grime 1983).

Interaction of light and fluctuating temperatures

The numbers of seedlings emerging on the light and dark thermogradient bars could be compared directly for each species as the soil was from the same sample and was tested simultaneously. The results demonstrate that at any given amplitude of temperature fluctuation germination was generally greater in the light than in the dark in all species. Nevertheless, stimulation of germination by fluctuating temperatures was evident in both dark and light, i.e. light could not entirely substitute for fluctuating temperatures.

These results were expected as seeds can develope a light requirement during burial (Wesson and Wareing 1969 (b), Taylorson 1970) and of course a light requirement is already present in freshly shed seeds of many species. Naturally buried seeds that are not exposed to light are therefore less likely to germinate at any given temperature than those which have been exposed to light, unless certain temperature regimes can completely substitute for the light requirement.

The noticeable effect of increasing temperature fluctuations on the light thermogradient bar can be explained in several ways. It is possible that this apparent effect is really an artefact, arising from limited penetration of light into the soil in the light treatment. The effect of this would be to superimpose a constant level of germination due to light on a variable level due to stimulation of germination by fluctuating temperatures in that portion of soil below the level of light penetration. The very different shapes of the light and dark germination curves in several species render this explanation unlikely. It is probable that the light spectrum would change with increasing soil depth even in soil with an open structure such as that on the bars (Wooley annd Stoller 1978), and there may be

some interaction between the far-red part of the spectrum and the amplitude of temperature fluctuations experienced by the seeds. Larger temperature fluctuations may be needed to overcome the inhibiting effects of far-red light even when seeds are very near the soil surface.

If the stimulation of germination by fluctuating temperatures in the light is a real effect then this is in contrast to the results of Thompson and Grime (1983) using harvested seeds. They found that a requirement for fluctuating temperatures in several species (e.g. Holcus lanatus and Poa annua) was present in the dark but abolished by light. It is therefore possible that during burial seeds may acquire not only a requirement for light (Wesson and Wareing 1969(b)) but also a requirement for fluctuating temperatures.

Ecological Implications.

The species tested on the thermogradient bars all possess large buried seed banks. This suggests that the harvested seeds were inhibited to some degree by darkness as species which germinate readily in darkness are unlikely to accumulate buried seeds. Once buried a further inhibition by darkness may lead to secondary dormancy (Wesson and Wareing 1969(b)) and some seeds may then have an obligate requirement for light, i.e. they have to be unearthed before they will germinate (e.g. Juncus acutifolius, Hypericum perforatum). The fate of other buried seeds is determined by their response to temperature fluctuations and the evidence shown here suggests that these responses may vary greatly between species.

Sensitivity to fluctuating temperatures as a depth sensing mechanism.

Diurnal temperature fluctuations are greatest near the soil surface and diminish rapidly with depth (Thompson 1977) and therefore sensitivity to temperature fluctuations in the dark is obviously capable of acting as a depth sensing mechanism. Most of the species tested do not germinate to any appreciable extent in the dark unless the amplitude of temperature fluctuations exceeds approximately 7°C and this would prevent the germination of deeply buried seeds. Since the ability of seedlings to emerge from depth is related to seed size (Thompson and Grime 1983) it would be expected that the smallest seeds should require the largest fluctuations. In fact the germination of species with very small seeds was almost completely prevented by darkness (e.g. Juncus acutiflorus, Hypericum perforatum and Epilobium tetragonum), irrespective of the amplitudes of temperature fluctuations. An exception to this was the germination of the extremely small seeds of Agrostis stolonifera, which was hardly reduced at all by darkness but was increasingly stimulated by fluctuating temperatures up to approximately 20°C. It is perhaps significant that this latter species is a grass, a family in which the ability to penetrate soil may be markedly modified by the peculiar morphology of the seedling.

While harvested seeds of species which germinate easily in darkness at constant temperatures usually have large seeds (Thompson and Grime 1983), nevertheless a requirement for temperature fluctuations in darkness was found in buried seeds of species with a wide range of seed sizes (including Rumex obtusifolius and Polygonum aviculare). This suggests that the ecological role of sensitivity to fluctuating temperatures is not just concerned with depth sensing by seeds.

Sensitivity to fluctuating temperatures as a gap-detecting mechanism.

Thompson and Grime (1983) mention several separate lines of circumstantial evidence suggesting that sensitivity to temperature fluctuations in darkness may act as a mechanism allowing buried seeds to 'detect' gaps in the canopy of foliage and litter. Milthorpe (1961) goes so far as to state that "it is reasonably certain that the establishment of plants from seed in vegetation occurs only in bare areas arising from the death of previous occupants or from incomplete coverage". It is obvious that this would have advantages for seedling establishment due to reduced competition from other plants for light, moisture and nutrients.

There is good evidence (Thompson 1977, Thompson, Grime and Mason 1977) that foliage and more especially litter may exert a very great insulating effect on the soil and that diurnal temperature fluctuations occurring in bare soil are much larger than those experienced by soil beneath a closed canopy. The results from the thermogradient bars suggest that different species might be adapted to germinating in different sized gaps. For example Holcus lanatus might exploit smaller gaps than Coronopus didymus or Poa annua. An experiment to investigate this by covering soil samples with an artificial litter layer and recording seedling emergence in relation to temperature fluctuations is described later in this chapter. These differences in germination response to temperature regime support the theory reviewed by Grubb (1977) which suggests that species which coexist in a particular plant community are likely to have distinctly different germination niches. This is discussed in Chapter 6.

Sensitivity to fluctuating temperatures as a moisture-sensing mechanism.

A rather unexpected feature of the results discussed here is the reduction in germination at high fluctuations found in several species, particularly in the dark. It is known that soil both warms and cools more rapidly when it has a low moisture content and field measurements of temperatures 2cm below the soil surface confirm that fluctuations of more than 20°C only occur after several days of drought (Fig. 4.9). A lack of response to high amplitudes of temperature fluctuation could therefore protect seeds from germinating when there would be insufficient moisture for the seedlings to survive. Several workers have found that soil moisture plays an important role in restricting the germination of Poa annua (Benjamin 1974, Roberts and Potter 1980). This species showed a marked supression of germination at mean fluctuations of 24°C when tested on the thermogradient bars. Conversely, species that were stimulated to a great extent by large fluctuations (e.g. Rumex obtusifolius, Plantago major and Polygonum aviculare) produce large (and, in the case of Rumex obtusifolius, deep rooted) seedlings that would perhaps be relatively less affected by a mild drought.

Field experiments

Some of the predictions about buried seed response to temperature regime made from tests on the thermogradient bars were investigated for soil samples kept in more natural environmental conditions on the roof of Plymouth Polytechnic. It was expected, from previous tests, that smaller numbers of seedlings would emerge from soil that experienced smaller amplitudes of temperature fluctuations and that the presence or absence of light would have a large effect on some

species even under a fluctuating temperature regime. Soil was therefore subjected to various mean amplitudes of temperature fluctuations in the light and the dark for a period of five months.

Method

The problems experienced when recording seedling emergence in permanent quadrats in the field due to the 'patchy' horizontal distribution of seeds in the soil (described in Chapter 4) were overcome by using soil which was collected and prepared in the same way as that used for the thermogradient bar tests which involved thorough mixing (details in Chapter 2). The effects on seedling emergence caused by changes in soil moisture were eliminated by watering the soil regularly to maintain it at field capacity. This soil was collected from Site 2 which was a derelict pasture (see Plate 4.1) and from Site 3 which was a field containing a daffodil crop (see Plate 4.2). The soil from each site was subjected to three different treatments (Fig. 5.10 and Plate 4) each containing three replicates.

Each replicate contained the same weight of dry soil (360g) which was spread uniformly (4-5mm deep) on a bed of perlite (25mm deep) in a small plastic seed tray and this was contained within a larger seed tray so that the edges of the soil layer could be insulated by more perlite. Excess soil moisture drained through the seed trays. Treatment 1 allowed light to reach the soil which was covered by one layer of green netting (2mm mesh size) to prevent the soil and perlite from blowing away. The soil in Treatment 2 remained in darkness as it was covered by a layer of sterile sand (3-4mm) and two layers of green netting. The amplitude of diurnal temperature fluctuations experienced by the soil was reduced by approximately 2°c due to the

sand, compared with the soil in Treatment 1. The soil in Treatment 3 was covered both by sand and by a layer of foam rubber (12mm thick) which reduced the amplitude of temperature fluctuations by approximately 8°c compared with the soil in Treatment 1.

The actual temperatures near the surface of the soil layer in each treatment were recorded at hourly intervals throughout the experiment by thermocouples attached to a 'Grant' temperature recorder and the daily maximum and minimum temperatures were noted from the recorder chart (see Figs. 5.11-5.16). The difference between these values was calculated to show changes in the amplitude of temperature fluctuations; this is also illustrated in Figs. 5.11-5.16. Seedlings were identified, counted and removed at approximately ten day intervals. The sand layer enabled the two dark treatments to remain in darkness during seedling counting and removal. The numbers of seedlings of the three most common species from each site are shown for each treatment on the acetate overlay sheets attached to the above mentioned figures.

FIG. 5.10

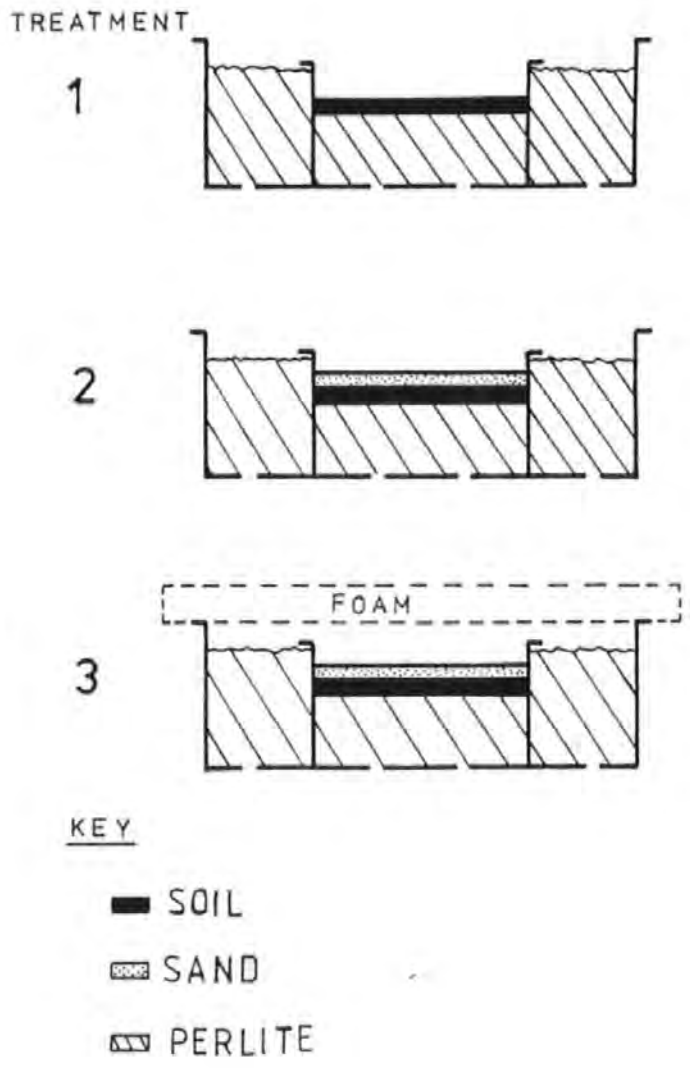


Fig. 5.10

Treatments for the experiment carried out on the roof of Plymouth Polytechnic. See 'Field Experiments' section in the text (Page 116) for details.

FIGS. 5.11 - 5.16.

Temperatures recorded just below the surface of layers of soil on the roof of Plymouth Polytechnic (1983), (see Plate 4, back of thesis). The soil received the various insulating treatments shown in Fig. 5.10, Page 119.

Each acetate overlay shows the total numbers of seedlings that emerged from naturally buried seeds in the soil sample. Seedlings were recorded and removed at approximately ten day intervals.

FIGS. 5.11 and 5.14. 'Foam' soil treatment (soil covered with 3-4 mm. sand and 12 mm. foam rubber).

FIGS. 5.12 and 5.15. 'Dark' soil treatment (soil covered with 3-4 mm. sand).

FIGS. 5.13 and 5.16. 'Light' soil treatment (bare soil).

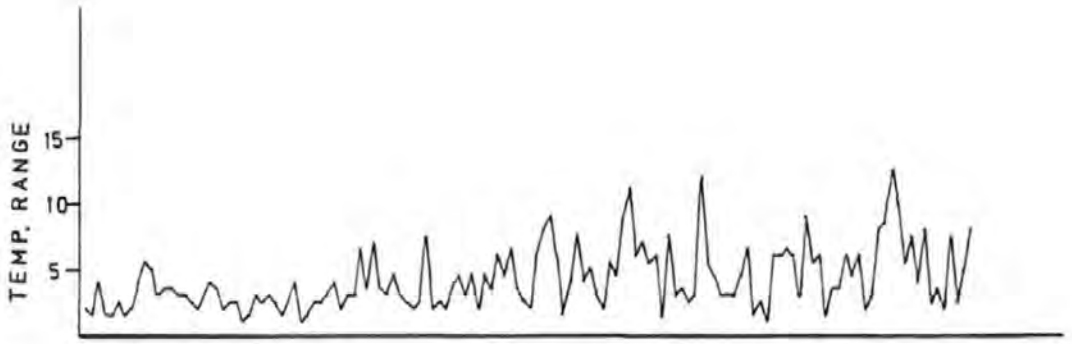
FIGS. 5.11 - 5.13 Soil collected from derelict pasture site, (site 2).

FIGS. 5.14 - 5.16. Soil collected from daffodil field site, (site 3).

KEY Holcus lanatus —— Coronopus didymus ·····

Rumex obtusifolius - - -

FIG. 5.11 (foam)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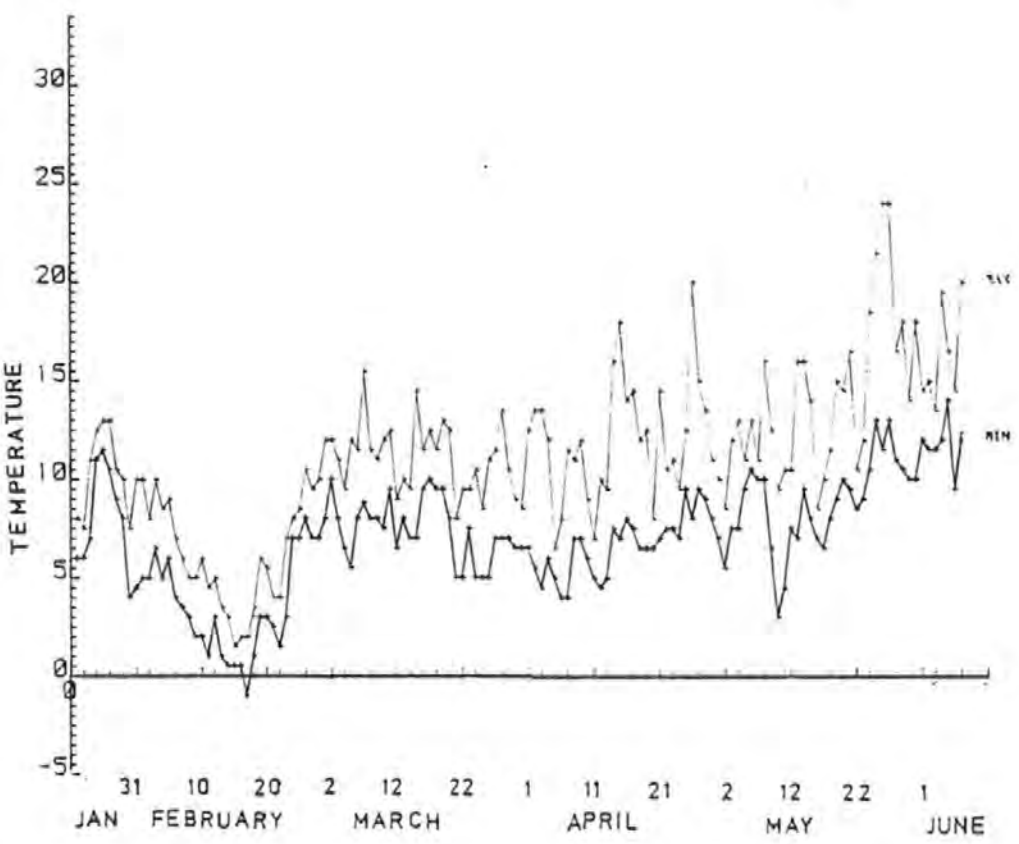
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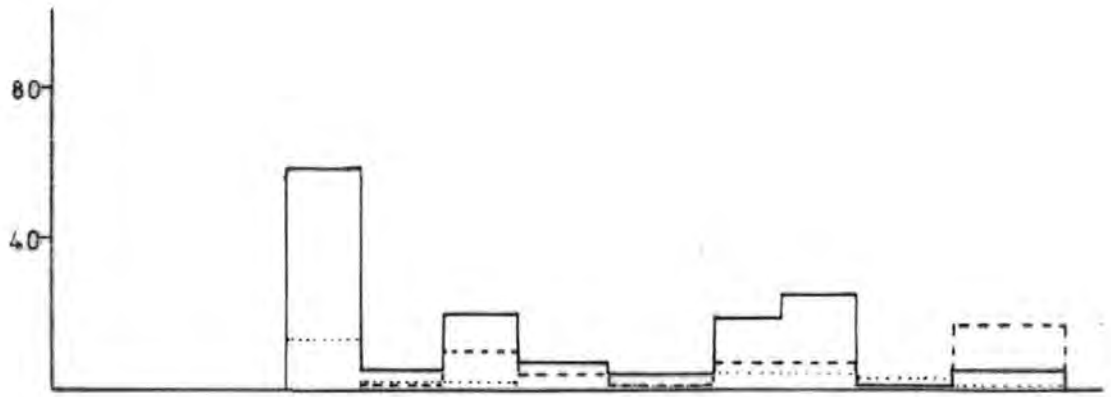
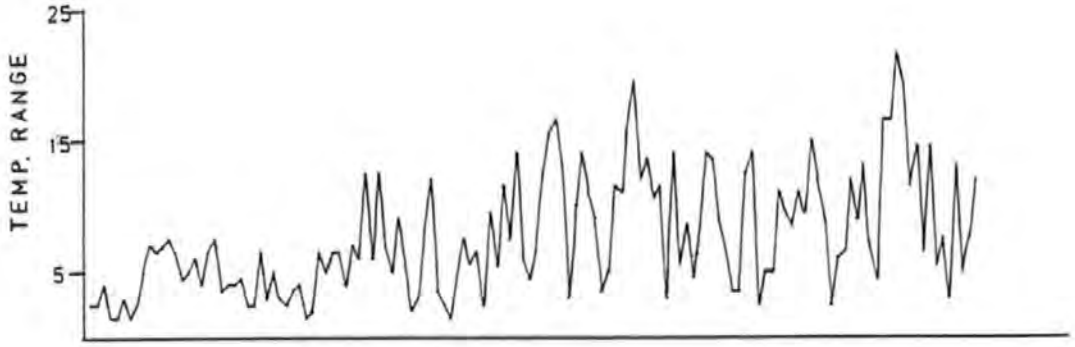


KEY Holcus lanatus —— Coronopus didymus ·····

Rumex obtusifolius - - -

FIG. 5.12 (dark)


TEMP. RANGE
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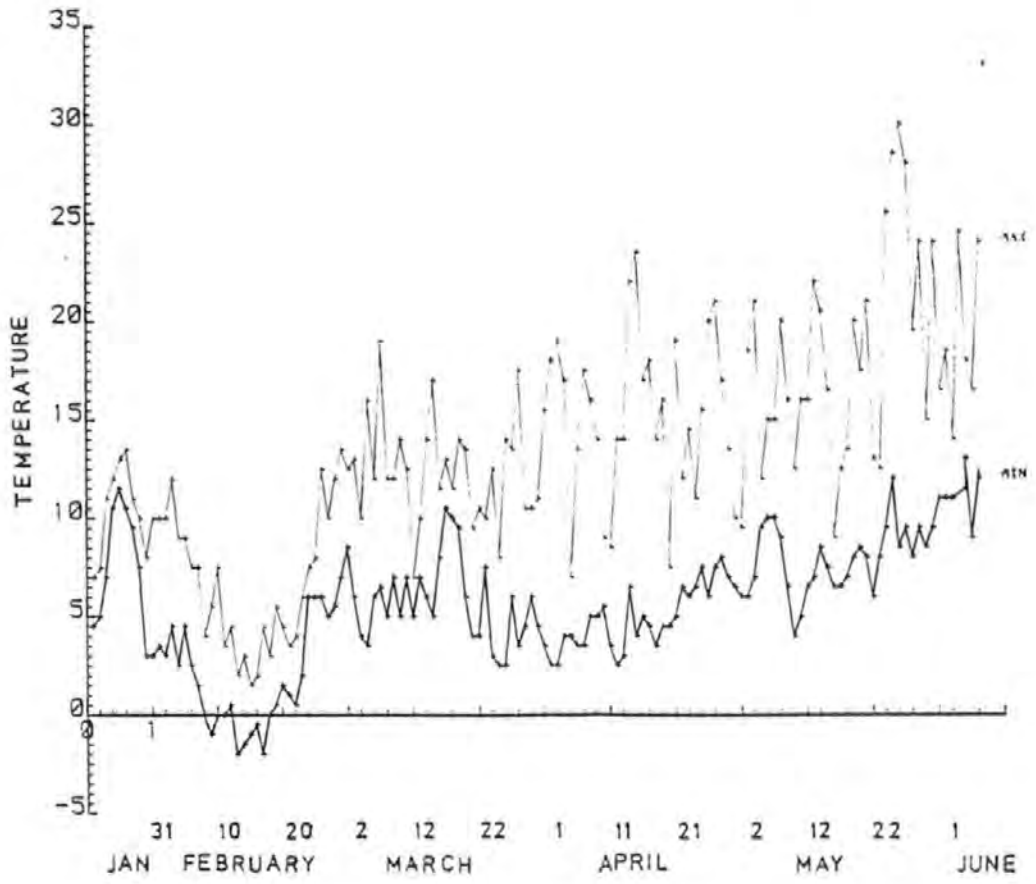
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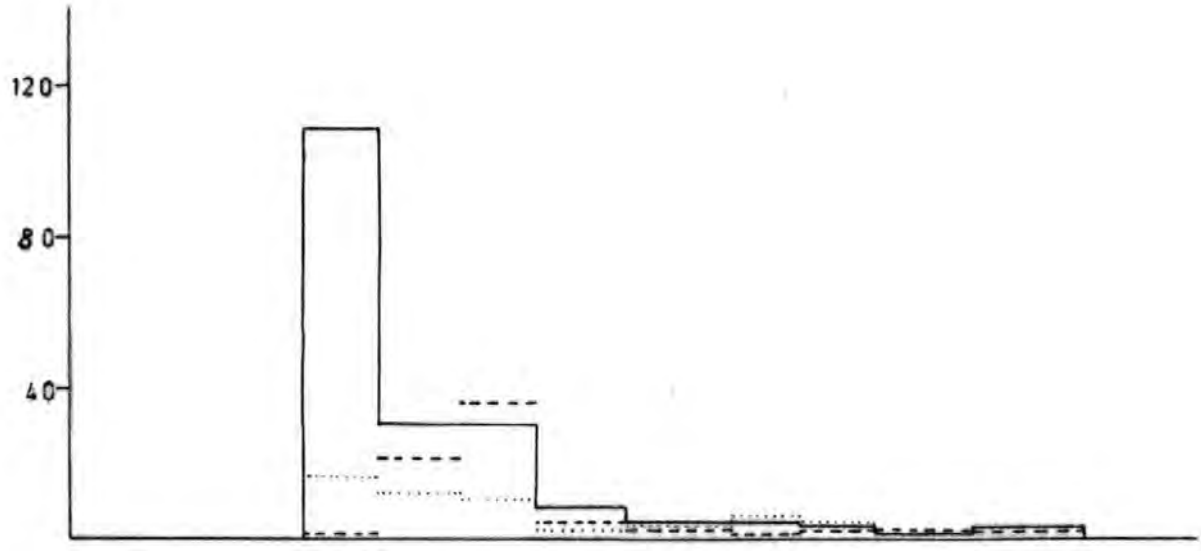
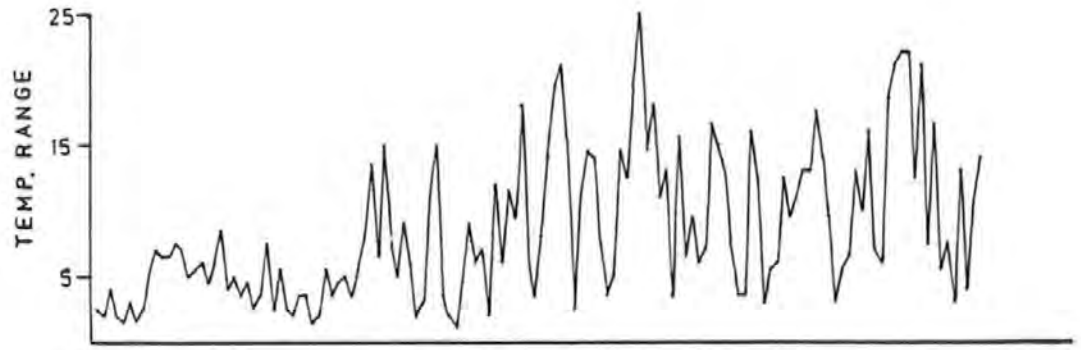
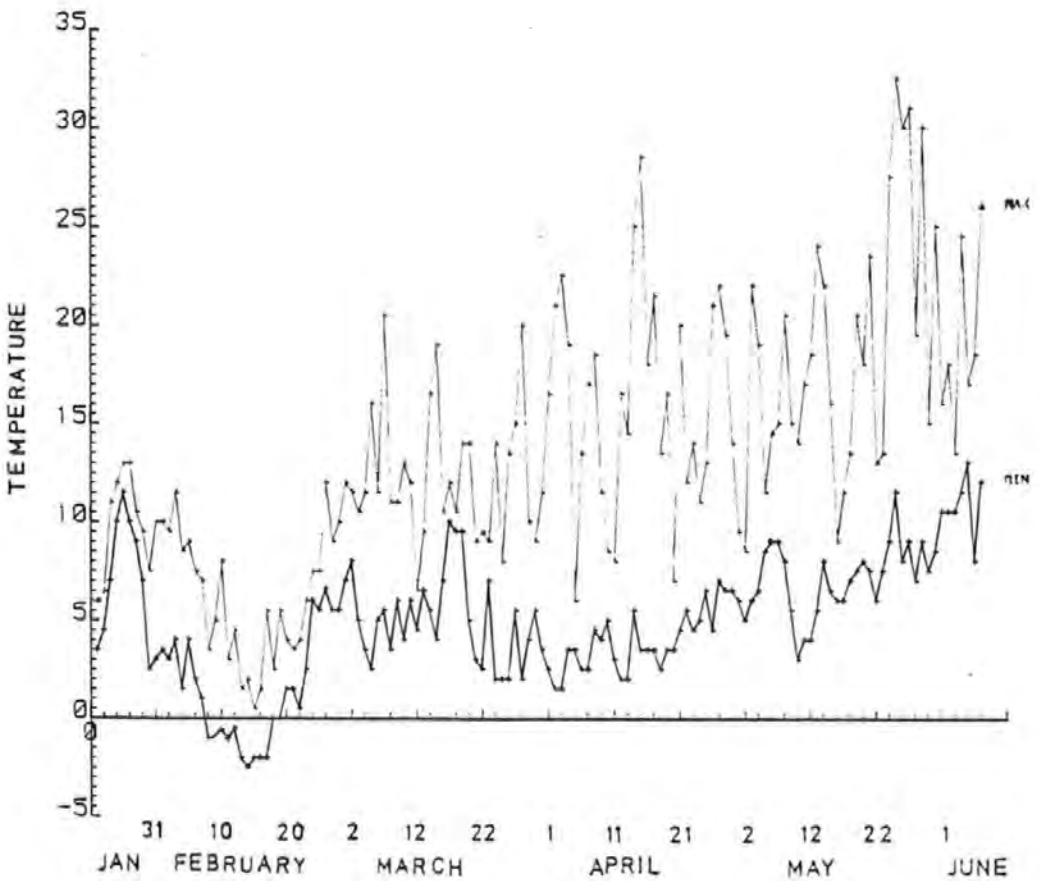


KEY Holcus lanatus ——
Coronopus didymus .....
Rumex obtusifolius - - -

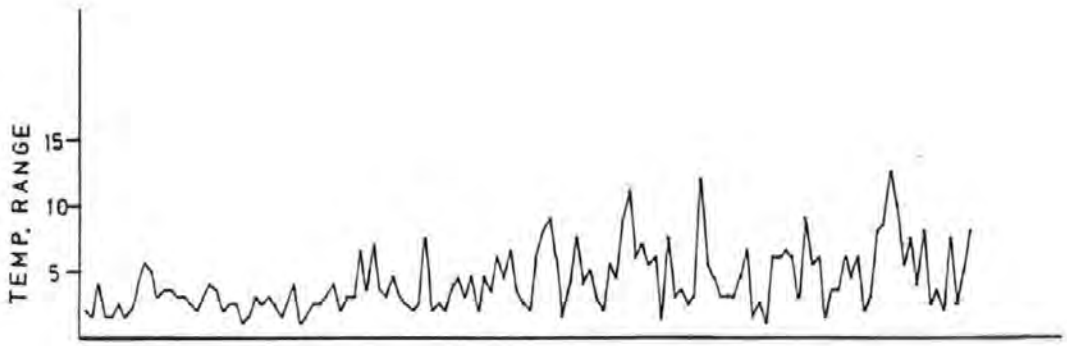
FIG. 5.13 (light)





KEY Epilobium tetragonum ——
Polygonum aviculare ----
Papaver rhoeas .....

FIG. 5.14 (foam)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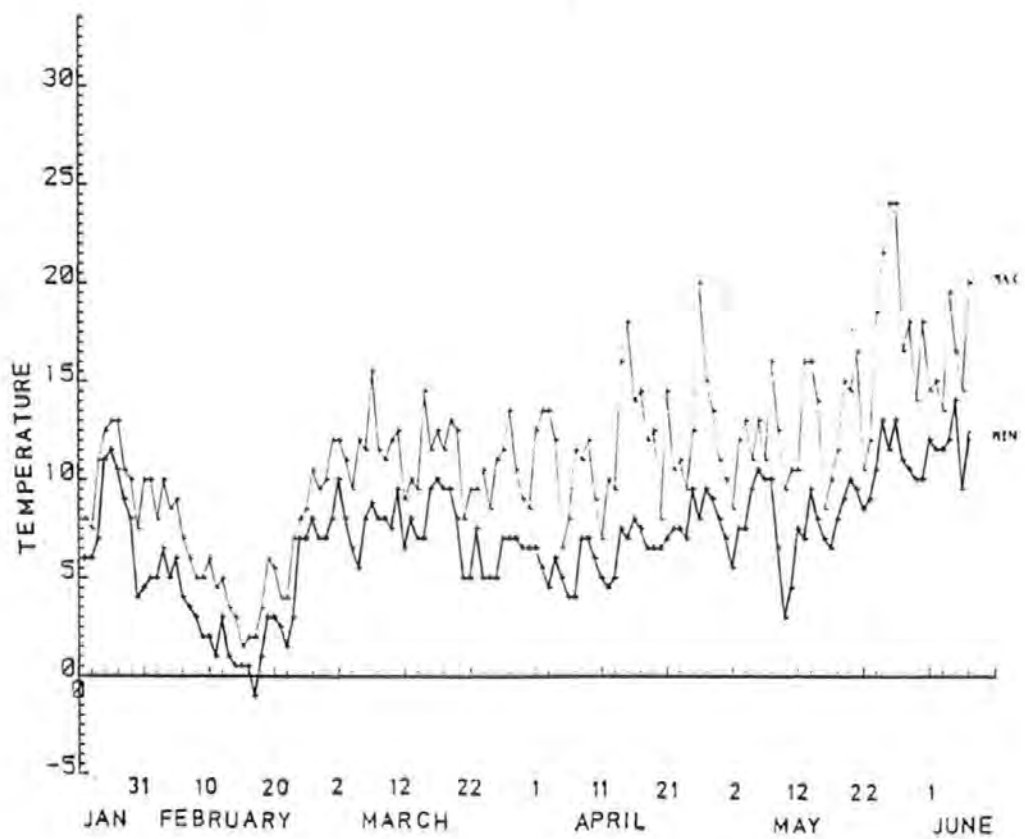
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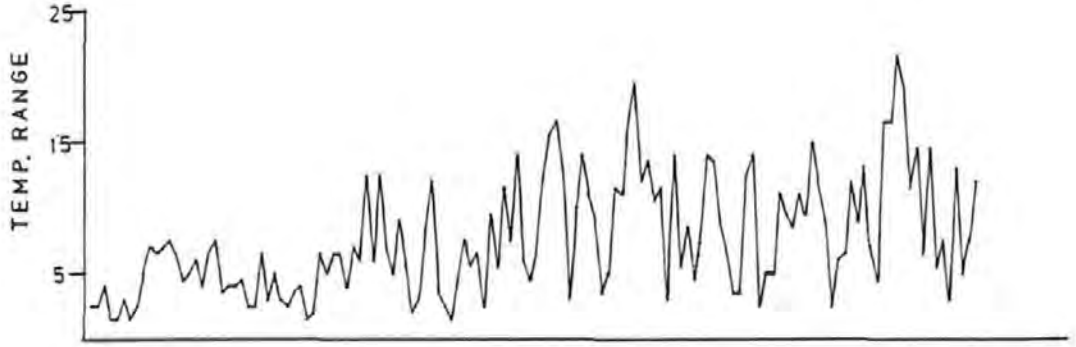
FIG. 5.15 (dark)


TEMP. RANGE
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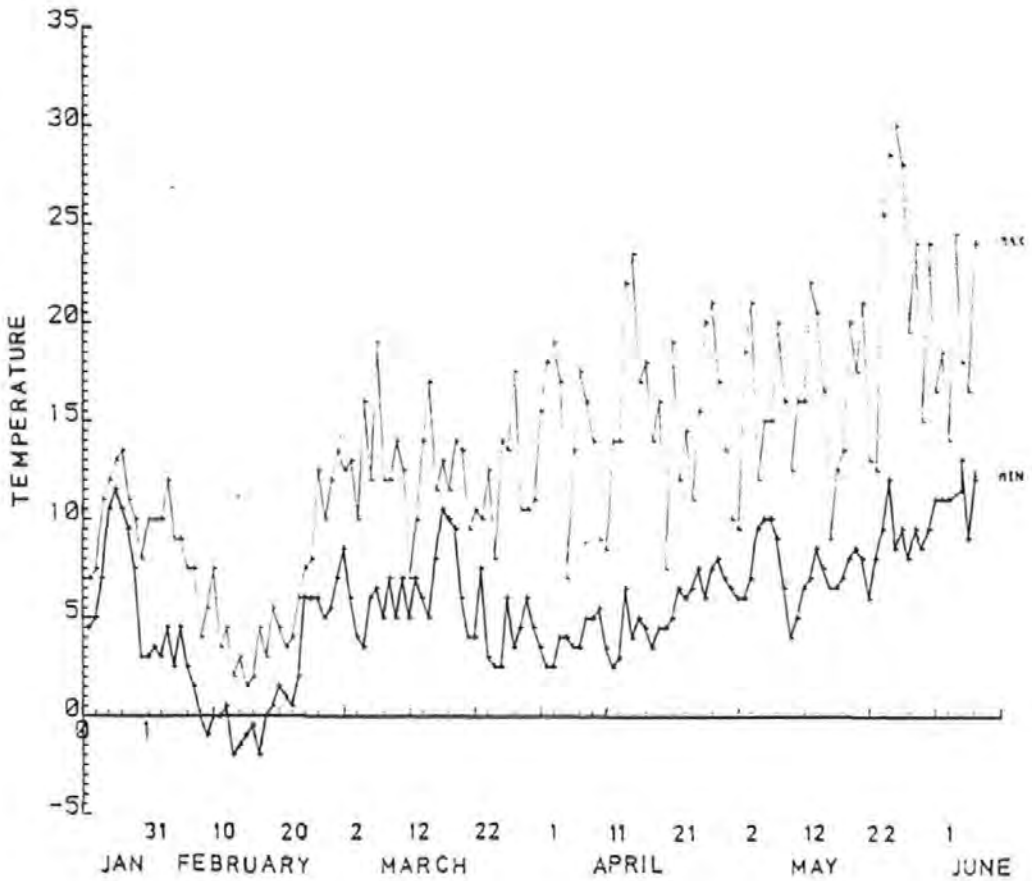
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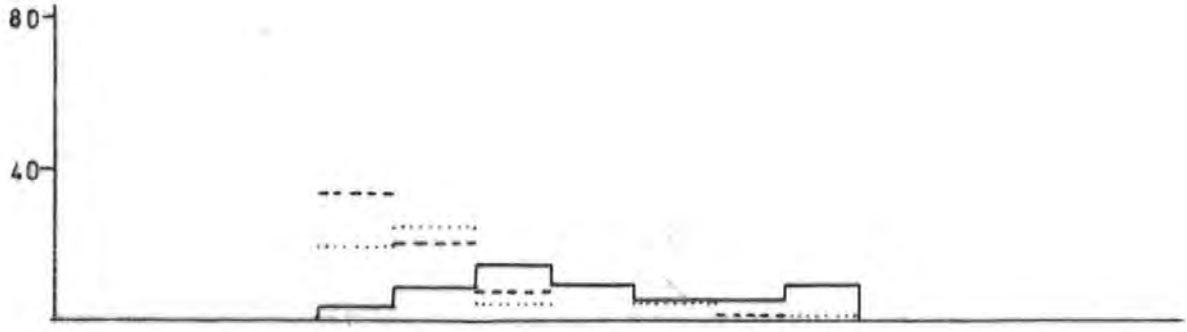
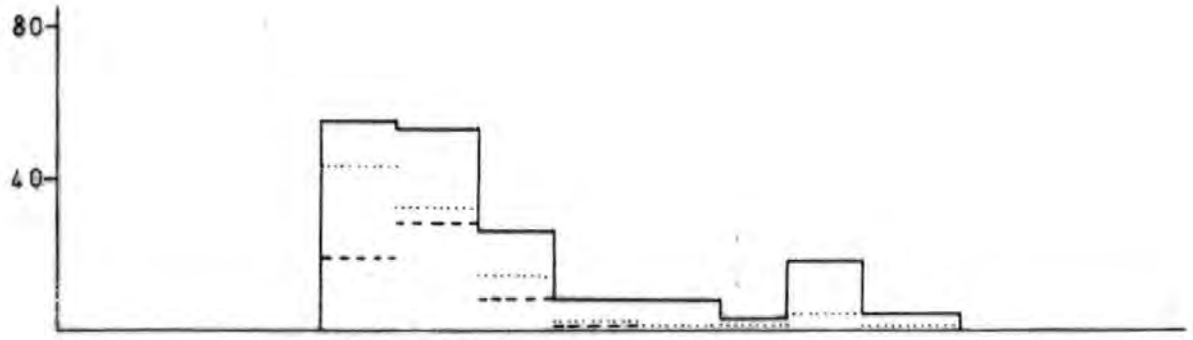


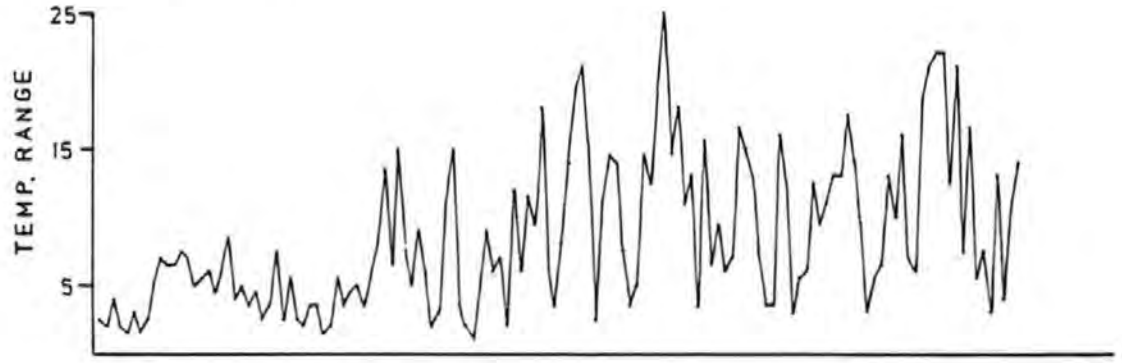
KEY Epilobium tetragonum ——
Polygonum aviculare ----
Papaver rhoeas .....

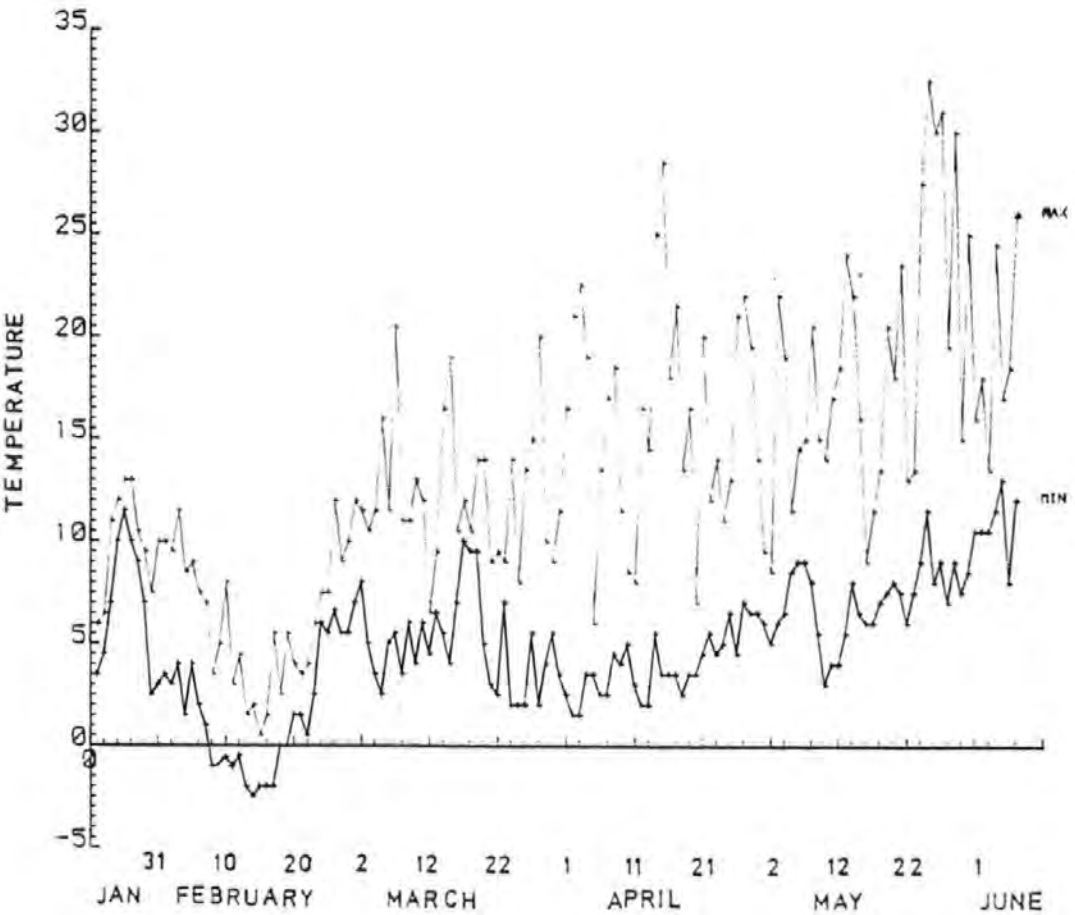


KEY Epil'obium tetragonum —— Papaver rhoeas ·····

Polygonum aviculare ----

FIG. 5.16 (light)





Results and dicussion

In order to simplify the following discussion, Treatment 1 will be referred to as the 'light' treatment, Treatment 2 as the 'dark' treatment and Treatment 2 as the 'foam' treatment. The temperature graphs show that the range of temperature fluctuations in the light and dark treatments only differed by approximately 2°c, the range being generally between 3-16°c in the light and 3-14°c in the dark after the first week in March. The range of temperature fluctuations in the dark and foam treatments differed by approximately 6°c, the range being generally between 3-14°c in the dark and 2.5-7.5°c in the foam treatment after the first week in March.

There was a cold period in February followed by a rise in minimum temperatures and then larger and more variable temperature fluctuations in the first week of March in all three treatments. No seedlings emerged in any treatment before the beginning of March which suggests that the cold period had a significant effect on breaking seed dormancy. It is not certain if large fluctuations were experienced before this date during the winter without causing a germination response as the temperatures were only recorded from the last week of January onwards. It is known that the winter of 1982-1983 was exceptionally mild and the period in mid-February may have been the only below freezing temperatures experienced.

There is evidence for the soil from Site 2 (the derelict pasture) that the viable seeds in the seed bank may have been almost exhausted after the initial flush of emergence in the light in March. Very few ungerminated seeds were recovered from the soil when it was examined at the end of the experiment. The total number of seedlings that emerged in the light treatment may therefore, be lower than expected when compared with the dark treatment (Table 5.2).

TABLE 5.2

Effect of light treatment over dark treatment on total numbers of seedlings.

SPECIES	TREATMENT			
	1 (LIGHT)	2 (DARK)	% OF TOTAL IN LIGHT	
Holcus lanatus	191	142	74.3	site 2
Rumex obtusifolius	70	45	64.3	
Coronopus didymus	52	24	51.9	
Total of all seedlings	413	293	70.9	
Epilobium tetragonum	175	53	30.3	site 3
Polygonum aviculare	56	61	108.9	
Papaver rhoeas	98	52	53.1	
Total of all seedlings	451	238	52.8	

TABLE 5.3

Effect of reducing the amplitude of temperature fluctuations by approximately 6°c in darkness on total numbers of seedlings.

SPECIES	TREATMENT			
	2 (DARK)	3 (DARK+FOAM)	% OF TOTAL IN DARK	
Holcus lanatus	142	95	66.9	site 2
Rumex obtusifolius	45	11	24.4	
Coronopus didymus	24	4	16.7	
Total of all seedlings	293	139	47.4	
Epilobium tetragonum	53	4	7.5	site 3
Polygonum aviculare	61	35	57.4	
Papaver rhoeas	52	21	40.4	
Total of all seedlings	238	93	39.1	

It can be seen from Table 5.2 that the effect of light on the total seedling emergence varied greatly between species as expected from tests on the thermogradient bars. For example, the emergence of Epilobium tetragonum (Fig. 5.4) would be expected to be greatly reduced in darkness whereas Holcus lanatus (Fig. 5.4) would be reduced to a lesser extent. The curves for Polygonum aviculare (Fig. 5.7) suggest that seedling numbers would be relatively lower in darkness, which was not the case in the field.

The total number of seedlings of most species was greatly reduced by the drop in amplitude of temperature fluctuations between the dark and foam treatments. The effect of these treatments could be predicted for each species tested in the dark on the thermogradient bars by the difference in the number of seedlings between the 9.6°c fluctuation section and the 14.4°c section (approximately equal to the mean amplitude of temperature fluctuations in the foam treatment and dark treatment respectively). The drop in numbers for seeds tested on the bars with smaller temperature fluctuations was particularly marked for Coronopus didymus (Fig. 5.3), Rumex obtusifolius (Fig. 5.7) and Polygonum aviculare (Fig. 5.7). There was a less significant drop in seedling numbers for Holcus lanatus (Fig. 5.4) and Epilobium tetragonum (Fig. 5.4). These observations are similar to the data in Table 5.3, the one exception being Epilobium tetragonum.

Apart from the light treatment, in which there was evidence that the number of viable seeds was rapidly depleted through germination, the emergence of seedlings tended to occur in irregular flushes. The initial flush in all treatments

correlated with the end of the cold period in February and subsequent flushes showed some correlation of timing between the three treatments for the six main species. However there was little

correlation between the six species in the patterns of seedling emergence and consequently the relationship between emergence and environmental factors needs to be considered for each species separately.

Polygonum aviculare

Tests on Polygonum aviculare on the thermogradient bars suggested that most seeds would germinate after fluctuations of approximately 12-20°c in the light and 12°c upwards in the dark (Fig. 5.7). One would expect this species to germinate early in spring but rapidly have secondary dormancy induced as the maximum temperatures increased (Courtney 1968, Roberts and Potter 1980). An examination of the field results (Figs. 5.14,5.15 and 5.16) showed very little emergence after early April in the light and dark treatments but the emergence period was extended into the end of April in the foam treatment. This could be explained if secondary dormancy is induced in these seeds by maximum temperatures greater than approximately 15°c.

Papaver rhoeas

Papaver rhoeas showed a similar pattern to Polygonum aviculare though a small number of seedlings emerged in May. It was particularly responsive to chilling in the light. Insufficient seedlings emerged on the thermogradient bars for valid statistical analysis, so no comparison between treatments can be made.

Epilobium tetragonum

Following the chilling stimulus it seems that virtually the whole population of buried Epilobium tetragonum seeds was capable of immediate germination even at low temperature fluctuations, as long as

they were exposed to light. In the dark this initial massive flush of germination was quite absent and germination may have been triggered by periods of consecutive large temperature fluctuations. The increase in germination caused by a series of temperature fluctuation cycles rather than isolated temperature shifts is discussed in Chapter 7. As expected from the thermogradient bar tests there was very little germination of Epilobium tetragonum in the foam treatment where darkness was combined with small temperature fluctuations. In the light treatment germination of this species appears to be reduced by maximum temperatures greater than 25°c (e.g. end of April and end of May).

Holcus lanatus

The response of Holcus lanatus to chilling is unexpected (Grime et. al. 1981) and there seem to be a proportion of seeds which will germinate after chilling even with low fluctuations and no light which is in contrast to the germination response of seeds tested on the thermogradient bars without a chilling pretreatment (Fig. 5.4). This initial germination flush at the end of February was very similar in size in both the dark and foam treatments and therefore seems to be independant of temperature fluctuations,but its size was increased by light. After this flush the separate flushes of Holcus lanatus in the dark and foam treatments were correlated with periods of large temperature fluctuations (>10°c in foam and >15°c in dark). This is supported by the observation that fluctuations in the dark treatment are >10°c most of the time and the background level of germination in the dark is similar to the peaks in the foam treatment.

The pattern of seedling emergence for this species is quite different in the light from the other two treatments, presumably

because of exhaustion of seed reserves after the very large initial flush. There is also some evidence of exhaustion of seed reserves in the dark treatment because the final peak at the end of May is very low compared with that in the foam treatment.

Coronopus didymus

Coronopus didymus appears to respond to chilling both in the light and dark but subsequent flushes do not correlate with larger temperature fluctuations or to particular absolute temperatures. The small number of seedlings make it difficult to draw any conclusions for this species.

Rumex obtusifolius

Unlike other species, there is no flush of germination of Rumex obtusifolius immediately after chilling but this may indicate a longer interval between the stimulus and the response rather than a lack of response. The size and location of germination peaks after chilling are clearly correlated with fluctuating temperatures both in the light and the dark.

In the dark treatment Rumex obtusifolius was the most responsive of the three species recorded from the derelict pasture soil to the very large fluctuations (>15°c) in mid-May. However this may only indicate its more gradual depletion from the seed bank compared with the other species. There is again evidence for rapid germination of the majority of seeds in the light treatment in early Spring because, unlike Polygonum aviculare, there is no evidence for rapid induction of secondary dormancy by soil warming. This type of germination response would be predicted from the tests on the thermogradient bars (Fig. 5.7) where there was very little germination at amplitudes of

approximately 5°c but the number of seedlings increased rapidly to a maximum at approximately 14°c.

CHAPTER 6

THE MAINTENANCE OF SPECIES-RICHNESS IN PLANT COMMUNITIES.

Involvement of regeneration

Harper (1967) states that the existence of natural diversity implies that the struggle for existence is not regularly forced to decide between stronger and weaker brethren ----- and that the struggle between some forms living in the same area is either evaded or does not occur. In plants it is not easy to see how a group of species which all require the same basic food requirements (i.e. light, water, carbon dioxide and mineral nutrients) may possess sufficiently diverse biologies to prevent a best species from excluding all others.

Experiments showing that a balanced mixture of even-aged pairs of species can be maintained for long periods of time have been explained either by a balance of intraspecific versus interspecific competition (Harper 1967) or limitation of the two species by different factors (Harper, Clatworthy, McNaughton and Sagar 1961). For example, on some soils nitrogen is the primary limiting factor for grasses, but phosphorus and potassium limit the growth of legumes (Thurston 1969). Grubb (1977) suggests that the explanations listed above do not go far towards explaining the indefinite coexistance of many species in species-rich communities.

There is divergence of opinion on whether groups of species with strongly similar habitat niches (i.e. the physical and chemical limits tolerated by the mature plant in the natural habitat) are found together. Whittaker (1975) gives the impression that this is not a general feature of vegetation but Grubb (1977) gives examples of very similar adult groupings. Whichever view is taken the possibilities for different plant niches within a community are greatly increased when the different requirements for regeneration of each species (ie. the replacement of the individual plants of one generation by those of

the next) are taken into account. All stages in the regeneration-cycle are potentially important sources of variation and include the production of viable seed, dispersal in space and time, germination and establishment of the immature plant. Examples of plant communities in which co-existence is thought to be facilitated by differences in the regeneration niche include Plantago media and P. lanceolata in basic grassland (Sagar and Harper 1964) and Ranunculus acris, R. bulbosus and R. repens in certain pastures (Sarukhan and Harper 1973).

There are many relevant reports concerning the establishment of seedlings in the field but very little information is available on germination as such in the field --- the optimum conditions for this stage in the life-cycle can be quite different from those for seedling establishment.

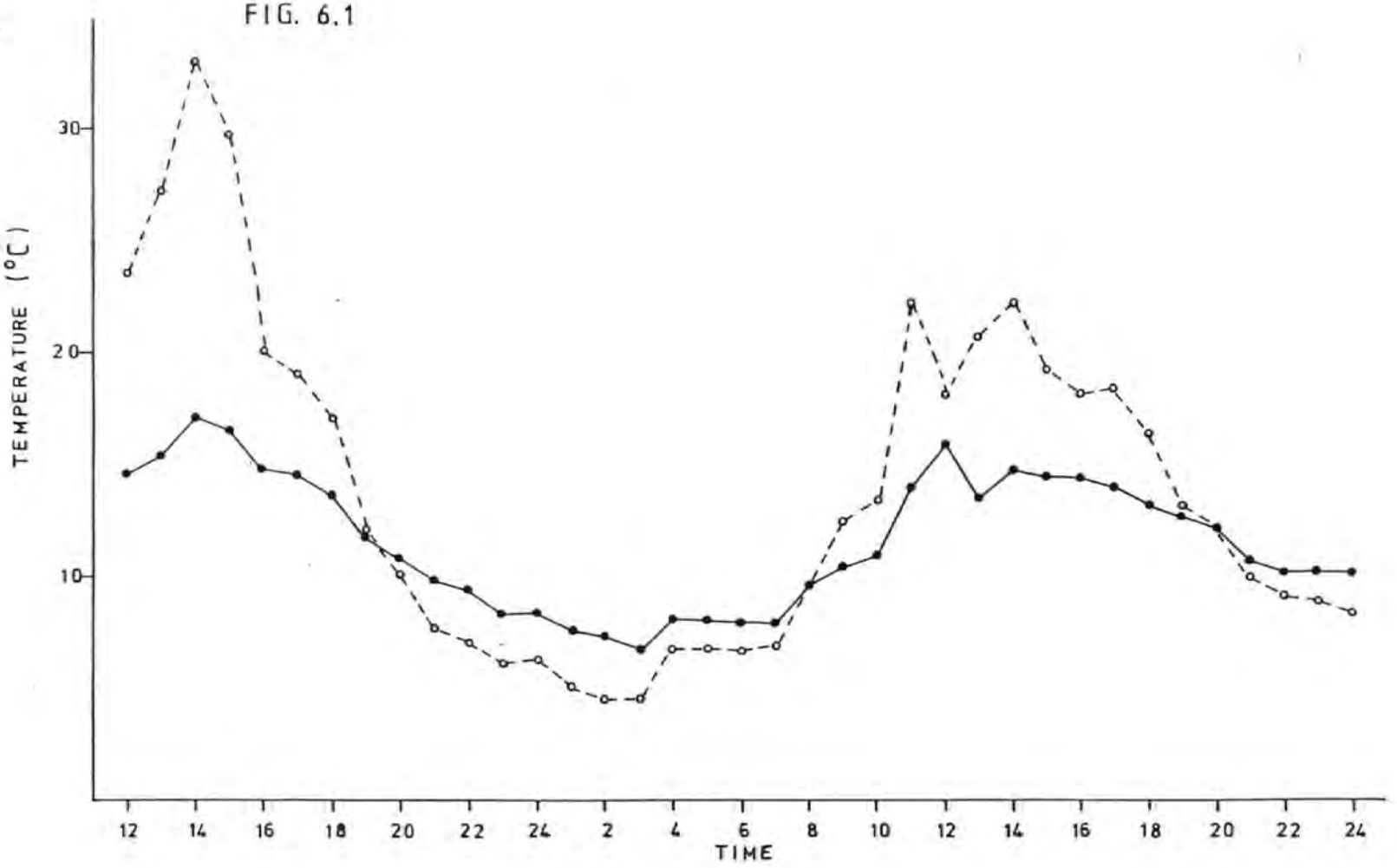
Possible involvement of temperature fluctuations

Results from tests on the thermogradient bar apparatus (Chapters 4 and 5) have shown that seed germination can be affected by the amplitude of temperature fluctuations to which seeds are exposed. The characteristics of temperature fluctuations in surface soil layers would depend largely on the nature of the vegetation and/or litter covering the soil. Measurements recorded at the derelict pasture site (Plate 4.1) on 21-4-82 showed large temperature fluctuations at the soil surface beneath sparse vegetation and very little litter. In contrast to this, the fluctuations were greatly reduced beneath dense grass and litter though not eliminated completely (Fig. 6.1).

FIG. 6.1.

Temperatures recorded at the derelict pasture site on 21-22 April 1982. On the soil surface beneath sparse vegetation (O------O) and beneath dense grass (●——●).

FIG. 6.1


TEMPERATURE (°C)
30
20
10
12
14
16
18
20
22
24
2
4
6
8
10
12
14
16
18
20
22
24
TIME

Gaps in the surface vegetation would increase the amplitude of temperature fluctuations in the soil depending on their size and time of creation. Gaps formed in different habitats would contain various depths of litter which would affect both the temperature and the light at the soil surface. Gaps containing no litter (e.g. those formed in regularly cultivated fields) are simulated by the light thermogradient bar whereas those containing large amounts of litter (e.g. neglected pasture or woodland) are simulated by the dark bar.

The interspecific differences in response to the temperature and light regimes on the bars which have been noted in previous chapters suggest that different species could be adapted to regenerating in different types of gaps. If this aspect of the regeneration requirements is important in maintaining species richness at a particular site, one might predict that there would be distinct differences between the germination responses to temperature regime of species contained in the same soil sample. This would be particularly likely if the adult plants had similar habitat preferences.

Experimental Method

In order to test the above hypothesis soil samples which contained appreciable numbers of seeds of two or more species were tested on the thermogradient bars. The mean number of seedlings of each species that emerged in the five ranges of temperature fluctuations were recorded, expressed on a per unit area basis and then transformed to a percentage of the mean total number of seedlings in each replicate (see Chapter 2 for a detailed explanation of the experimental methods). These percentages were plotted against the mean amplitude of temperature fluctuations experienced in each section (Figs 6.2 - 6.4). Fig 5.1 shows the absolute temperatures experienced

on the thermogradient bars. The use of percentage emergence data for each species allows the temperature response patterns to be compared, but interspecific variation in total numbers of seedlings may be great and the possible significance of this is discussed later.

The sites from which the soil was taken included fields which were disturbed at least once a year, an old pasture which had not been disturbed for several years, and a coppiced woodland which had been cleared the previous year. Some of these sites are illustrated in Plates 4.1, 4.2 and 4.3.

FIG. 6.2.

The emergence of Coronpus didymus (●·····●); Rumex obtusifolius (O——O); and Holcus lanatus (x-----x) from naturally buried seeds in soil tested in the light (a) and in darkness (b) on the thermogradient bars.

Both soil samples were collected on 4.11.81.

FIG. 6.2

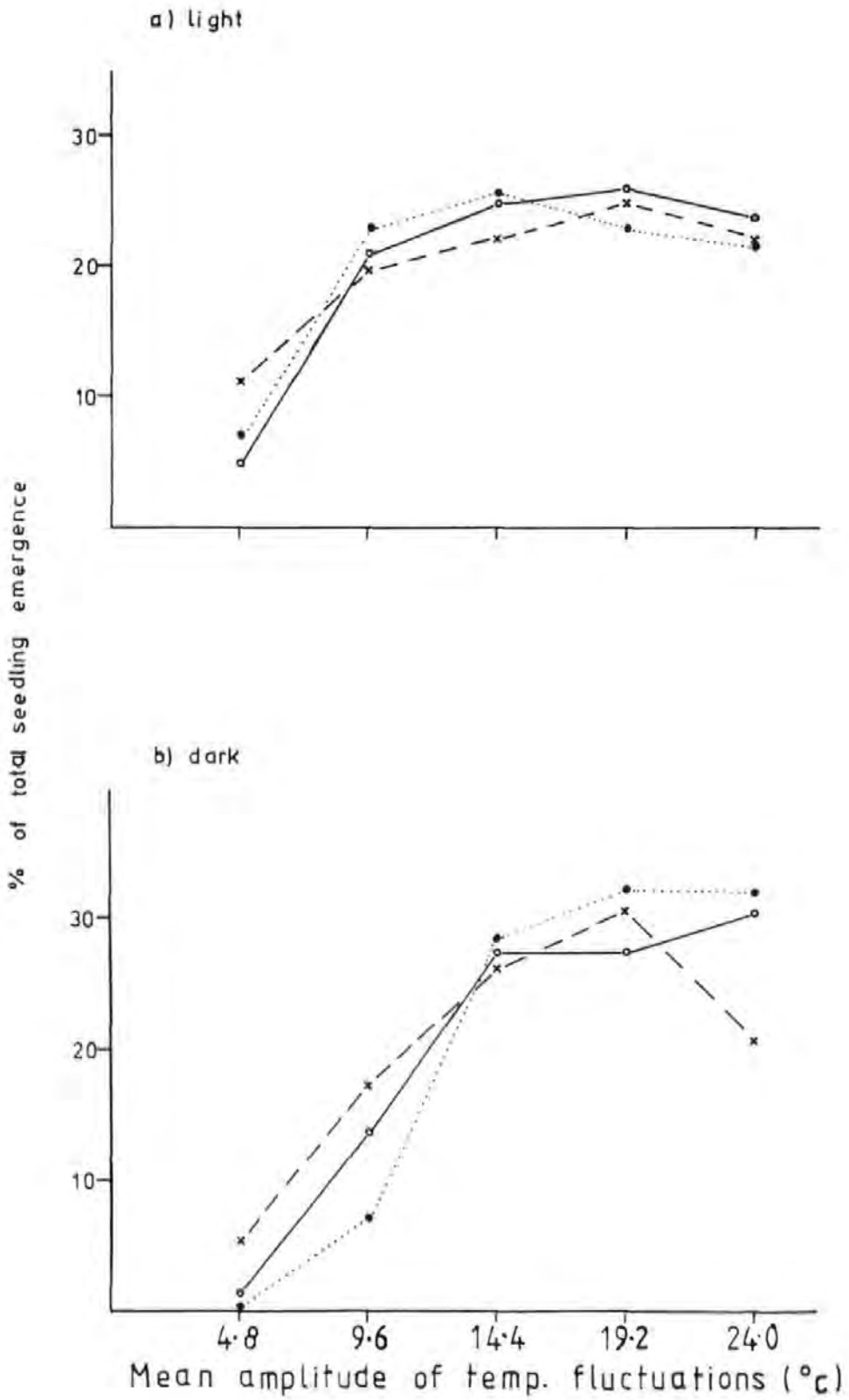

Derelict pasture 4·11·81
a) light
30
20
10
b) dark
30
20
10
% of total seedling emergence
4·8
9·6
14·4
19·2
24·0
Mean amplitude of temp. fluctuations (°c)

FIG. 6.3.

The emergence of *Coronopus didymus* (x - - - x), *Cardamine hirsuta* (O———O), *Epilobium tetragonum* (□-·-·-□) and *Veronica arvensis* (O·····O) from naturally buried seeds in soil tested in the light on the thermogradient bars.

FIG. 6.3

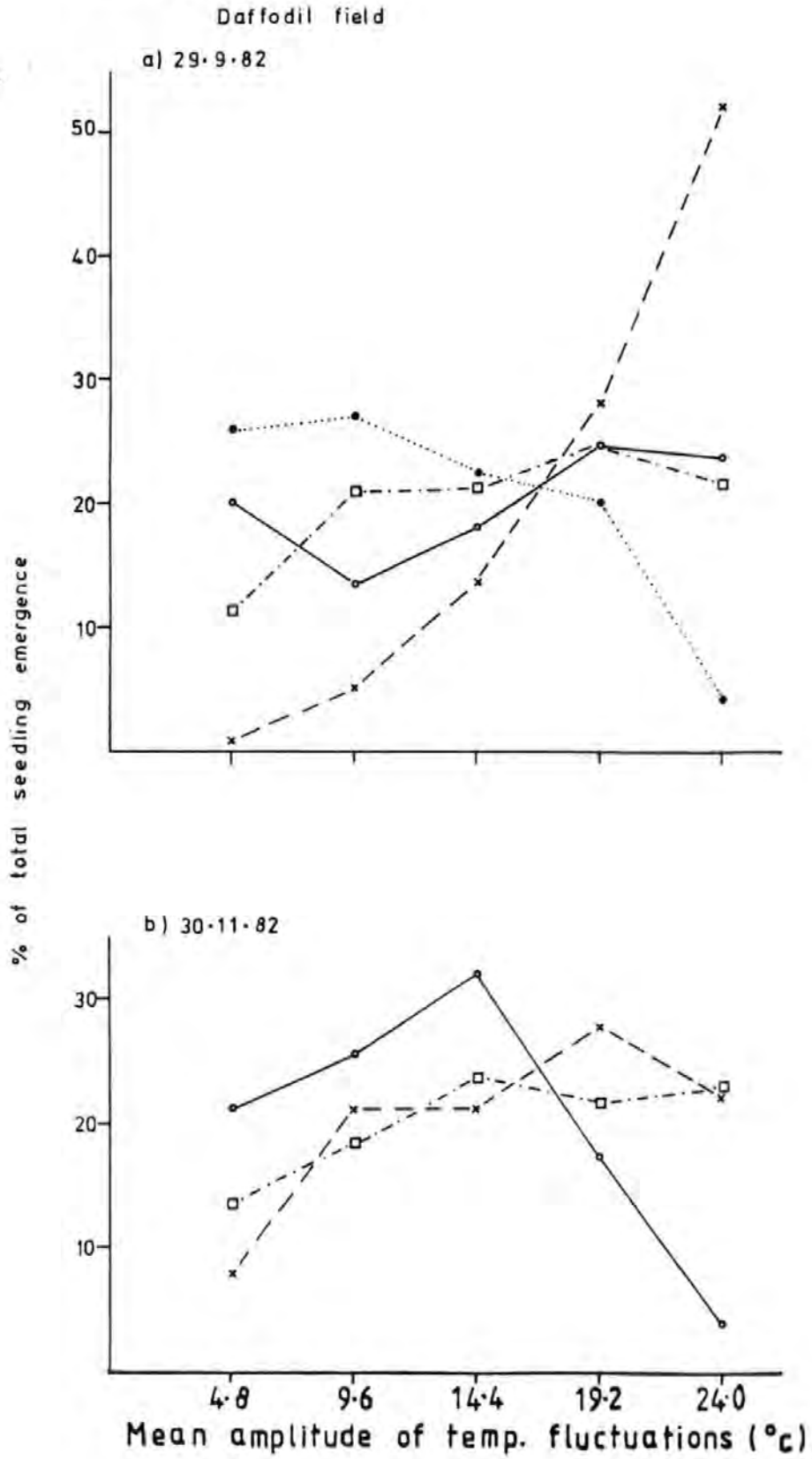

Daffodil field
a) 29·9·82
50
40
30
20
10
% of total seedling emergence
b) 30·11·82
30
20
10
4·8
9·6
14·4
19·2
24·0
Mean amplitude of temp. fluctuations (°C)

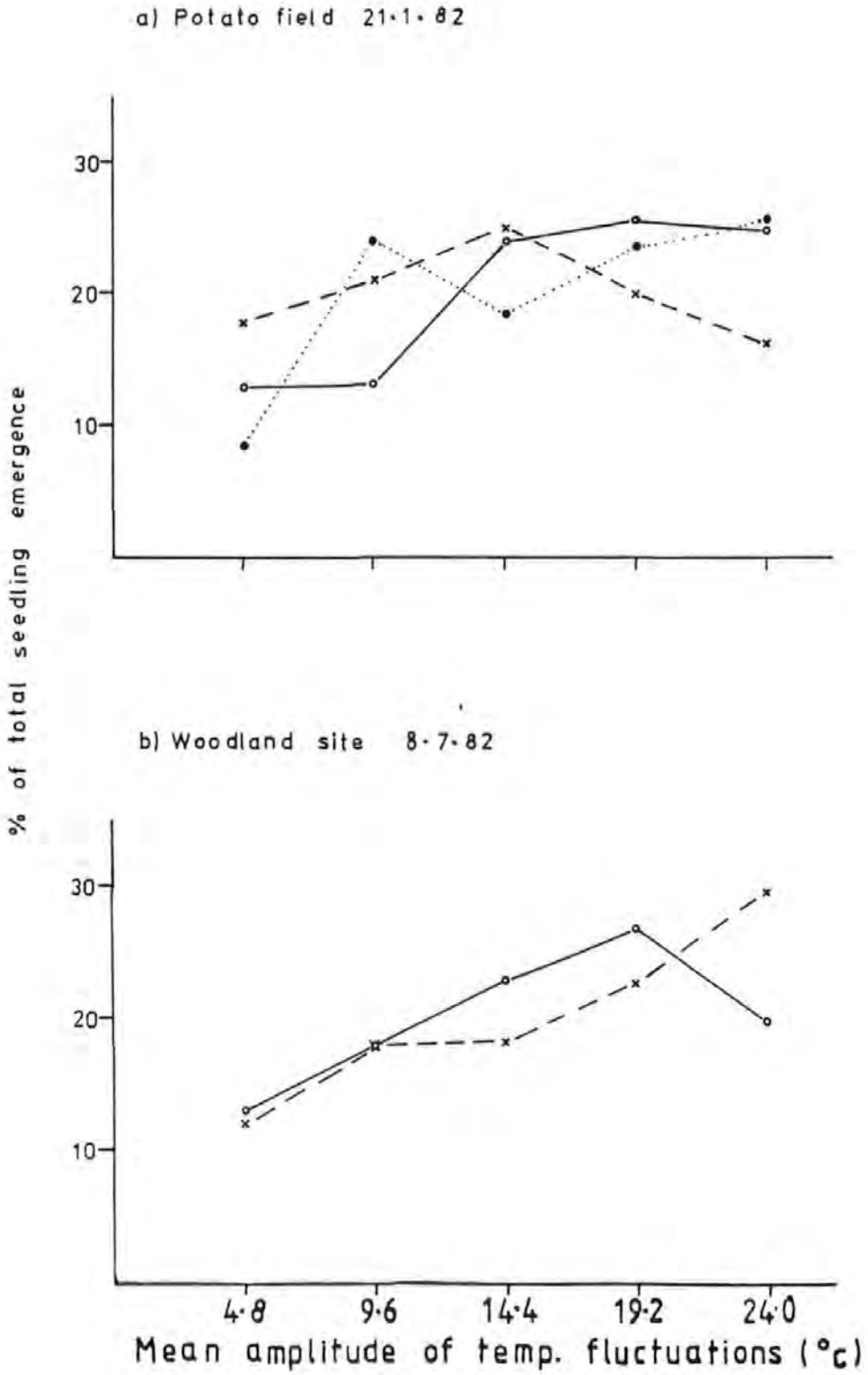
FIG. 6.4 (a).

The emergence of Chenopodium album (O——O), Spergula arvensis (O·····O) and Stellaria media (x- - -x) from naturally buried seeds in soil tested in the light on the thermogradient bars.

FIG. 6.4 (b).

The emergence of Digitalis purpurea (O——O) and Hypericum perforatum (x- - -x) from naturally buried seeds in soil tested in the light on the thermogradient bars.

FIG. 6.4


a) Potato field 21·1·82
30
20
10
b) Woodland site 8·7·82
30
20
10
% of total seedling emergence
4·8
9·6
14·4
19·2
24·0
Mean amplitude of temp. fluctuations (°c)

RESULTS AND DISCUSSION

The derelict pasture site

The results from the derelict pasture site (Fig 6.2) were interesting as it had been cultivated for daffodils fifteen years previously. The present vegetation consists mainly of Holcus lanatus, Rumex obtusifolius and Rubus spp. but when tested on the thermogradient bars seedlings of species associated with the daffodils emerged, e.g. Anagallis arvensis, Epilobium tetragonum, Cardamine hirsuta and Coronopus didymus as well as large numbers of the species found in the present vegetation.

The response of the three main species in the seed bank at this site to temperature fluctuations in the light is very similar, but these conditions would rarely arise in the field even in gaps as there is generally a relatively thick litter layer (Plate 4.1) which would exclude light from the soil surface. The results from the dark thermogradient bar suggest that germination of Holcus lanatus in small gaps (small temperature fluctuations) would be favoured compared with Rumex obtusifolius and particularly with Coronopus didymus. In very large gaps with temperature fluctuations of approximately 24°C and above this trend would be expected to be reversed, with a larger proportion of Coronpus didymus and Rumex obtusifolius emerging compared with Holcus lanatus.

Observations of vegetation in this field over several years have shown that Holcus lanatus has become more dominant and species diversity has been reduced as the canopy has become more closed and litter has built up. The results in Fig. 6.2(b) suggest that germination ecology has played a part in this increasing dominance by Holcus lanatus, although of course other factors such as the vigorous vegetative spread of Holcus lanatus may have been equally important.

There is still a possibility of new Rumex obtusifolius plants becoming established in larger gaps such as those formed by mole hills (King 1975).

The effect of gaps of different sizes containing variable amounts of litter (simulated by subjecting seeds to fluctuating temperatures in darkness) on seedling emergence was also investigated for soil from this site (see detailed discussion in Chapter 5). The gaps were simulated by covering the soil samples with a thin layer of sand and in one treatment, a layer of foam which reduced the mean amplitude of temperature fluctuations by approximately 6°C. A brief summary of the effect of these treatments on seedling emergence is shown in Table 6.1. The drop in temperature fluctuations caused a much greater reduction in Rumex obtusifolius seedlings than Holcus lanatus which is in agreement with the field observations and thermogradient bar data mentioned above.

TABLE 6.1

Effect of reducing mean amplitude of temperature fluctuations by approximately 6°c, in darkness, on total seedling emergence.

SPECIES	TREATMENT		
	DARK	DARK+FOAM	% OF TOTAL IN DARK
Holcus lanatus	142	95	66.9
Rumex obtusifolius	45	11	24.4
Coronopus didymus	24	4	16.7
Total of all species	293	139	47.4

The daffodil field site

In the daffodil field (Fig.6.3) there was clear evidence that different mean temperature fluctuations favoured different species. The soil collected on 29.9.82 suggested that Cardamine hirsuta and Veronica arvensis have an advantage over Coronopus didymus in small gaps or sheltered sites but Coronopus didymus has a great advantage in large gaps or exposed sites. One might therefore expect to find most Coronopus didymus seedlings between the rows of daffodils but more of the other species close to the bulbs where they are sheltered by the leaves (Plate 4.2).

There are likely to be marked seasonal changes in the proportions of seedlings emerging at this site as Veronica arvensis only emerged in autumn and Coronopus didymus showed a rapid change in response to temperature regime between the end of September and the end of November 1982. These results may be explained by the fact that Veronica arvensis is a winter annual in which secondary dormancy is induced by low temperatures. In the related winter annual Veronica hederifolia the onset of induced dormancy is well advanced by December and complete by January (Roberts and Neilson 1982). It has already been suggested in Chapter 4 that fresh seeds of Coronopus didymus (mainly shed in autumn) show primary dormancy which is only overcome by large temperature fluctuations.

It is possible that the timing of gaps produced by various cultivations or herbicide treatments at this site helps to maintain the species mixture. In this way Avena ludoviciana, which germinates in the winter, is a serious weed of winter cereals but is almost totally destroyed by ploughing in early spring. Avena fatua, which

germinates in the spring, is a severe pest of spring-sown cereals (Thurston 1951).

The potato field and woodland sites

The results in Fig. 6.4 suggest that relatively more Chenopodium album and Spergula arvensis are likely to be found in large gaps compared with Stellaria media. Hypericum perforatum is more likely to germinate in large gaps than Digitalis purpurea. However it is questionable as to whether the interspecific differences in temperature response found in the potato field and woodland site would be great enough to cause significant changes in the proportions of seedlings in various microsites.

Different germination responses to temperature fluctuations is only one of a variety of germination requirements which can ensure that seeds of different species germinate in sites that differ in time and space. Further potentially important variables include the speed of germination under a given set of conditions and the response to different soil moisture contents. Appreciable differences in the temperature responses of germination have been found in groups of species with very similar habitat-tolerances and reproductive strategies, e.g. among the winter annuals found on cliffs and sand-dunes in Europe and North America (Newman 1963, Baskin and Baskin 1971). The conclusion from Went°s experiments (1949) on desert annuals was that the composition of the final association or community as represented by the mature plants depended on interspecific differences in germination responses to both temperature regime and rainfall.

Effect of relative seed numbers

Although there is evidence for an effect of interspecific differences in germination response to temperature regime on species diversity, other observations at the field sites suggest that this effect may be over-shadowed by other factors. For instance relatively subtle differences in the pattern of response to temperature fluctuations may be masked by large differences in the representation of species in the seed bank. Several sites showed that many more seedlings of one particular species emerged at all the temperatures experienced on the thermogradient bars (see Figs 5.2-5.8). For example, Epilobium tetragonum in the daffodil field (Fig 6.3), Chenopodium album in the potato field (Fig 6.4) and Digitalis purpurea at the woodland site (Fig 6.4). If initial seedling numbers controlled the number of mature plants at these sites then the above species would be expected to exclude the less numerous species.

Cavers and Harper (1967) sowed seed of various taxa of Rumex spp. into a variety of plant communities in an attempt to influence the population size of the mature plants. One of the taxa used in this experiment was a maritime variety of R. crispus, var. littoreus (Hardy) which is almost completely restricted to a narrow zone of the sea shore at the level of the highest tides. Its distribution was obviously not restricted by germination requirements as the number of seedlings of maritime Rumex crispus exceeded that of the other species (often by a very large margin) in every habitat in which seed was sown. The major differences between the success of the taxa at different sites were shown to lie not in germination but in the differential survival of the seedlings. This suggests that the relative numbers of individuals of different species at a particular site at the initial seedling emergence stage may have little relation

to the final proportions of mature plants at that site.

Effect of disturbance

In general the role of the regeneration niche in maintaining species richness in plant communities seems likely to depend critically on the frequency and severity of disturbance. In the relatively undisturbed, closed communities the outcome of competition between established plants will largely determine the relative abundance of species. Equally in highly disturbed habitats there will be abundant open space most of the time and probably little opportunity for different species to specialise in gaps of different types and sizes. The greatest potential for regeneration niche differentiation will arise in habitats suffering moderate intensities of disturbance, particularly where the occurrence of gaps is variable both spatially and temporally. Thus the regeneration niche is another factor likely to promote high diversity in habitats suffering intermediate frequencies and intensities of disturbance (Huston 1979). Such habitats will provide opportunities not only for species which are adapted to different types of gaps but also for species which rely mainly on vegetative expansion to maintain their populations. The two carboniferous limestone grasslands described in Thompson and Grime (1979) are good examples of habitats of this type. In the present study a number of considerations, including the practical one of finding seed banks dense enough to give statistically valid results on the thermogradient bars, restricted the choice of sites to less diverse ones containing mainly 'weedy' species.

Horizontal distribution of seeds in the seed bank

Another factor likely to mask interspecific differences in response to temperature fluctuations is patchiness in the horizontal distribution of the seed bank, which will govern the presence or absence of particular species beneath different microsites. Silvertown (1981) suggests that differential dispersal of seeds into microsites with different amounts of leaf cover could result in the seed pool beneath large leaves becoming impoverished if the soil surface remained impenetrably covered for a sufficient length of time. This effect would be difficult to predict because of the role of animals in seed dispersal (McRill and Sagar 1973) and because of changes in vegetation cover caused by fluxes in plant population.

In an attempt to gain some impression of the magnitude of this patchiness in seed distribution a transect was cut across the vegetation at the derelict pasture and a row of quadrats (20cm x 20cm) were fixed on the bare soil. The total number of seedlings emerging from the four main species were recorded in each quadrat between 12-5-82 and 16-6-82 (Fig 6.5). The results give some indication of the great variability in the relative numbers of seeds of different species in the seed bank. Quadrats contianing a high density of a particular species may correspond to the previous location of parent plant that deposited a high concentration of seeds around its periphery (Greig- Smith 1979).

FIG. 6.5.

The total number of seedlings emerging between 12-5-82 and 16-6-82 in each of 21 quadrats (20cm x 20cm) spaced evenly along a 480 cm transect cut through the vegetation at the derelict pasture site.

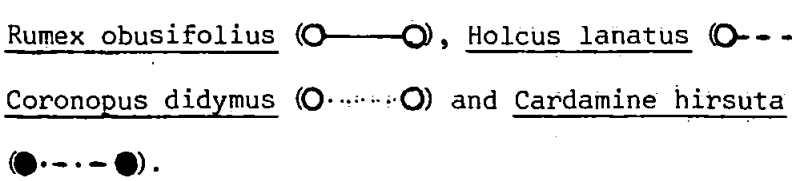
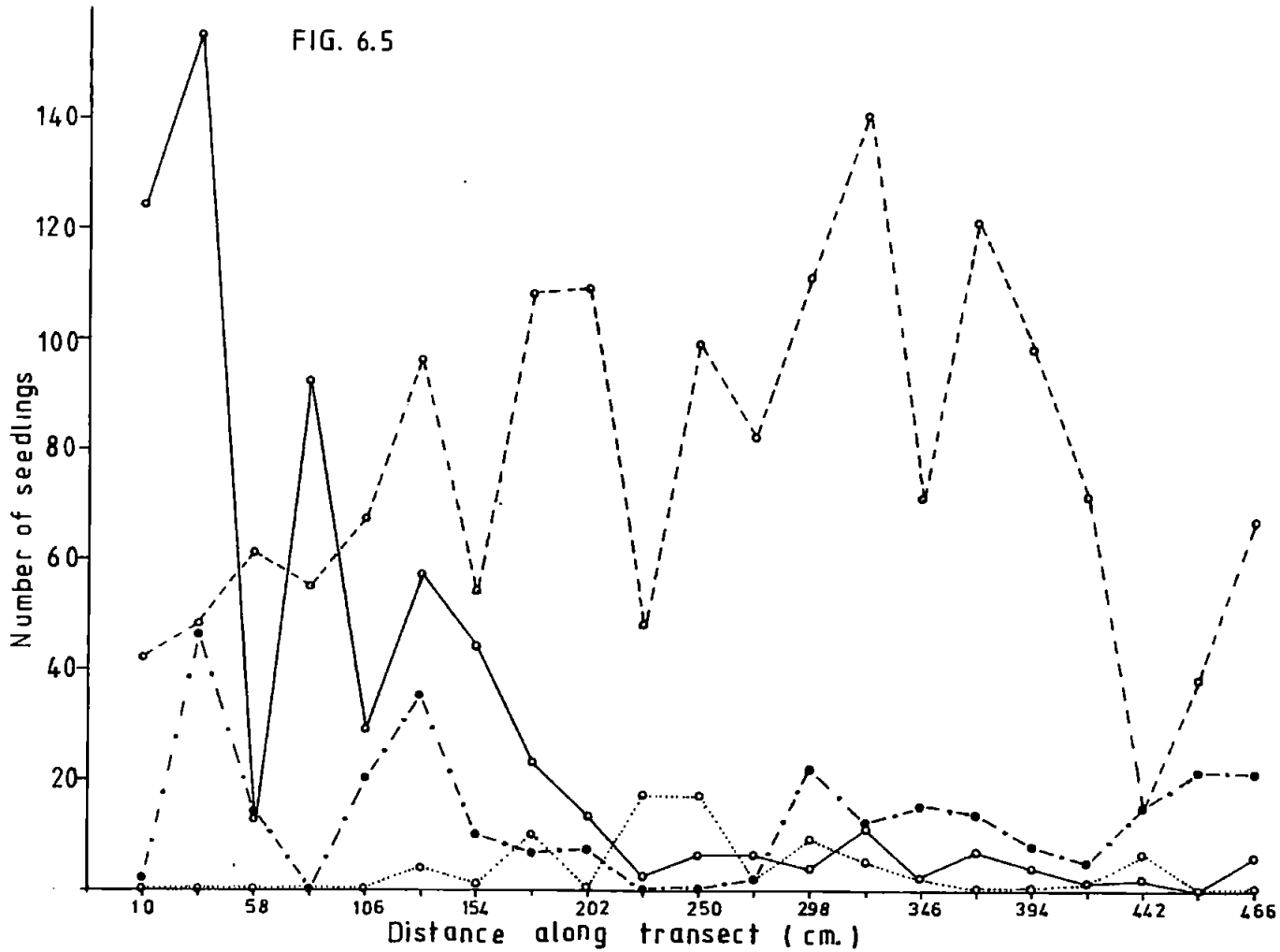


FIG. 6.5


Number of seedlings
140
120
100
80
60
40
20
10
58
106
154
202
250
298
346
394
442
466
Distance along transect (cm.)

These observations (Fig 6.5) suggest that even in gaps of similar sizes, containing similar amounts of litter and created on the same date there would be great differences in the numbers of different species emerging, depending on where the gap was created. Thus a particular size gap may be created that favours a given species in the seed bank but if no seeds of that species are present beneath the gap then no seedlings can emerge. This variability in the seed bank is therefore probably a major factor in maintaining diversity and tends to confound attempts to investigate germination requirements in the field.

Possible uses of the thermogradient bars in conjunction with field observations

There is obviously a need for observing seedling emergence from naturally buried seeds in the field. It would be useful to try and correlate emergence on a micro-scale with various environmental variables such as topography, leaf cover, soil temperature and soil moisture. This has recently been attempted by Silvertown (1981) and Grime and his colleagues (1982). Their reports show that it is necessarily detailed and time consuming work.

Silvertown (1981) observed the demography of natural populations of Reseda lutea and Anthyllis vulneraria in relation to local vegetation cover. Micro-variation in leaf cover within each quadrat (25cm x 25cm) was estimated in 625 one-cm square units. The conclusions were that Reseda lutea clearly responded to micro-spatial differences in leaf cover and was likely to regenerate only from microsites with low levels of shading. However, though Anthyllis vulneraria showed a similar tendency in one quadrat, the association was not shown in two others. Silvertown suggests that the greatest

source of 'error' in the correspondence between distribution of seedlings and micro-cover would be differential seed dispersal. This agrees with my observations (Fig 6.5).

By means of detailed field observations Grime and his colleagues (1982) have attempted to provide a quantitative analysis of the process of recolonization in vegetation gap of various sizes, shapes and seasons of origin in calcareous grassland. They found striking differences in patterns of germination between the north - and south facing slopes in the same valley. On the north-facing slope many more seeds germinated in gaps than in undisturbed vegetation. Such gap 'preference' was not apparent on the south-facing slope. Observations such as these provide data from which hypotheses and experimental work can be generated. However the complexity of factors involved in determining the regeneration of different species hampers the interpretation of such observations and it is difficult for hypotheses to be specific.

I therefore suggest that the thermogradient bars could be a useful tool to make field observations such as those described above more productive. Tests on the thermogradient bars can provide information about germination responses to different factors by eliminating or controlling much of the unwanted variation found in the field. Thus the soil preparation and testing techniques (see Chapter 2) allow variation in the horizontal distribution of seeds to be eliminated. The soil moisture and lighting can also be made more uniform. Not only can the responses to a gradient of temperatures be investigated but the apparatus can also be modified to produce a gradient of red:far-red ratios (Grime and co-workers 1982). Having built-up predictions about the germination responses of particular species these could then be tested by measuring environmental

variables at the field site and recording seedling emergence in relation to these factors. In fact this was carried out for several species of buried seeds. Predictions were made from tests on the thermogradient bars concerning germination responses to different amplitudes of temperature fluctuations (Figs 5.2 - 5.8). It was then possible to use these predictions to explain the observed emergence patterns of seedlings of several different species in a semi-natural environment (Figs 5.11 - 5.16).

More substantial evidence could eventually be collected concerning the relative importance of different environmental variables on the time and place of seed germination. This could lead to a quantitative model that would take many factors into account and provide a figure for the probability of a seedling of a particular species emerging in a given microsite (providing the relevant parameters of the microsite were known). This would be a useful management tool. Such a model has been created to predict competition and secondary succession at different sites in a temperate deciduous forest, (Botkin, Janak and Wallis, 1972).

SUMMARY

The field observations carried out in this survey suggest that at cultivated or recently cleared sites there are many potential microsites for weed seedling establishment and there is rarely competition between seeds for these sites. The seedling distribution is probably largely governed by the underlying pattern of seeds in the seed bank which is likely to be very patchy for each species (Fig 6.5). In these cases, particularly where seeds are exposed to light at the surface of gaps (i.e. no litter present), the responses of different species to temperature regime would be of minor importance

in maintaining species diversity. The timing of the appearance of gaps would be important where winter and summer annuals are present in the seed bank and some cultivations could be timed to avoid the peak periods of germination. The germination of certain species with light requiring seeds e.g. Epilobium tegragonum could be reduced by minimizing soil cultivations.

At sites where there is an almost continuous cover of vegetation with varying amounts of litter and less severe disturbance patterns it seems that differences between regeneration requirements could be much more important in maintaining species diversity.

In order to investigate predictions made from the thermogradient bars about the types of gaps that may favour the germination of one species over another at a particular site, long-term field observations are necessary. For any one plant community it would be necessary to record the size and date of formation of gaps that arise, temperatures and light intensity within the gaps and the spatial distribution of propagules of different species. Seedling emergence should be recorded in relation to these variables. It may eventually be possible to create a quantitative model to predict the probability of regeneration of different species at particular micro-sites.

CHAPTER 7

THE EFFECTS OF ENVIRONMENTAL STIMULI ON HARVESTED *RUMEX OBTUSIFOLIUS* SEEDS AND THE PERSISTENCE OF THESE EFFECTS UNDER VARIOUS STORAGE CONDITIONS.

Introduction

The experiments and field observations described in the previous chapters raised several questions concerning the dormancy characteristics of seeds. It was felt that these questions could best be investigated under specific controlled conditions. In particular it was hoped that laboratory experiments on harvested seeds might give an indication of the mechanisms underlying the phenomena observed in buried seeds. Nevertheless the results of such experiments must be intrpreted with care. The dangers of extrapolating the results from petri-dish experiments on stored seeds to seed responses in a field situation have been outlined in the introductory chapter of this thesis (Chapter 1) and are discussed in more detail by Koller (1964).

Particular stimuli which significantly affect the germination response of harvested seeds in the laboratory may have their effects masked by other interacting stimuli which are experienced by buried seeds in the field.It is known that many harvested seeds are in a state of primary (innate) dormancy whereas buried seeds are likely to have entered secondary dormancy which is induced when germination is inhibited. However it is stated in a recent review (Bewley and Black 1982) that no fundamental biochemical or physiological distinction has been recognised between the two dormancy states, so buried seeds and harvested seeds should respond to dormancy inducing or breaking stimuli in a similar manner. The results of the following experiments give guidelines for work that can usefully be carried out on seeds under more natural conditions.

Methods

Rumex obtusifolius seeds were used for these experiments as buried seeds of this species had been used extensively in previous

tests and the harvested seeds showed an obligate requirement for light at a constant temperature (18°c) and a large response to temperature fluctuations in the dark (Fig. 3.2). Harvested seeds were dried at room temperature, their outer coatings were removed by gentle abrasion and the seeds were separated from this chaff using the apparatus shown on Plate 3 (back of thesis).This apparatus is described in detail in Chapter 3. Seeds were stored in the dark at 5°c until needed.

Germination tests

The standard germination test involved counting out 25 seeds onto two layers of Whatman filter paper moistened with 4ml. of distilled water, in a petri-dish (5cm. diameter) which was then placed in an incubator at the desired temperature.

Six replicates were used for each treatment unless otherwise stated. Temperatures used for the tests were either a constant regime at 18°c or a diurnally fluctuating regime of ten hours at 25°c and fourteen hours at 9°c. The fluctuating temperature regime was known to stimulate the germination of naturally buried seeds on the thermogradient bars. The incubators were thermostatically controlled to within 1°c of the nominal temperature.

Unless otherwise stated all germination tests were carried out in darkness for 14 days. It is known that under certain conditions seeds can become highly responsive to brief light flashes (Gorski 1975) which commonly stimulate germination. Great care was therefore taken to ensure that seeds were not exposed to any light atall during the germination tests. Batches of petri-dishes for a particular treatment were placed in trays and covered with a layer of aluminium foil and a layer of thick black plastic. Germination was recorded at the end of a test where possible or, if necessary, by using a dim green 'safe'

light in a dark room. Where seeds in a particular experiment were dried between treatments the petri-dishes were opened in a dark growth cabinet where dry air could be blown across them. Germination was defined as emergence of approximately 1mm of radicle through the seed coat. Germination percentages of 98-100% could be obtained when seeds were imbibed in the light under a fluctuating temperature regime.

Light treatments

Unless otherwise stated "light conditions" refer to a daily 14 hour photoperiod given by a bank of warm-white fluorescent tubes in the growth cabinet. Brief "white" light treatments used the same lights.

An apparatus was designed to expose seeds to predominantly far-red light (Fig.7.1). A bank of three tungsten filament strip lights behind ruby red and deep blue cinemoid filters (obtainable from Rank Strand Electric Ltd.) were wired into a wooden box. The heat from these lamps was partly dispersed by blowing cold air across the apparatus and was also absorbed by a perspex tank containing cold water that was refilled frequently. The imbibed seeds in petri-dishes were placed beneath the perspex tank which was covered by white card to reflect the light from the lamps onto the seeds. The apparatus was covered by black light-proof material to prevent external light sources from affecting the spectrum under the apparatus (see Fig.7.2). The spectrum is comparable to that found under a vegetation canopy though the apparatus does transmit a higher proportion of red light than a growing wheat crop (McCartney and Unsworth 1976). The temperature beneath the apparatus was maintained at approximatey 22°c.

FIG. 7.1.

Side view of the apparatus used to subject seeds to far-red light.

FIG. 7.2.

The light spectrum recorded beneath the apparatus shown in Fig. 7.1.

FIG. 7.1

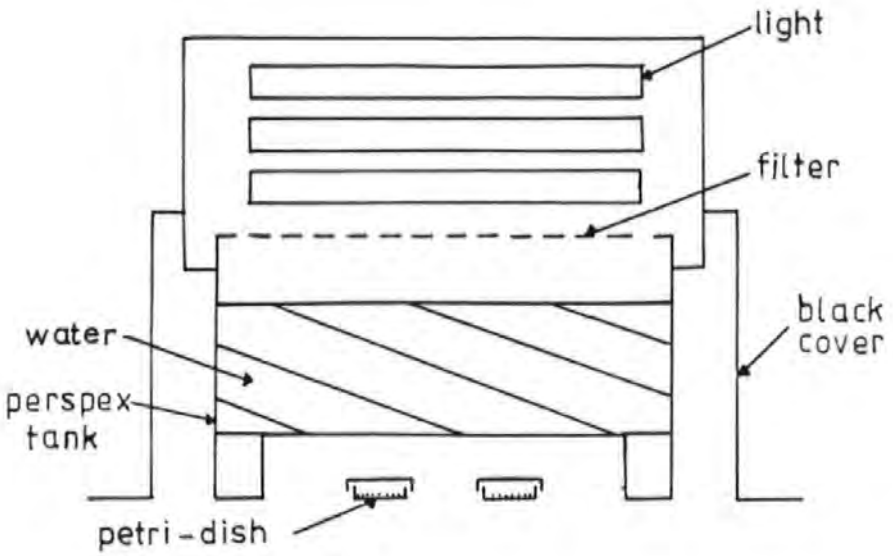
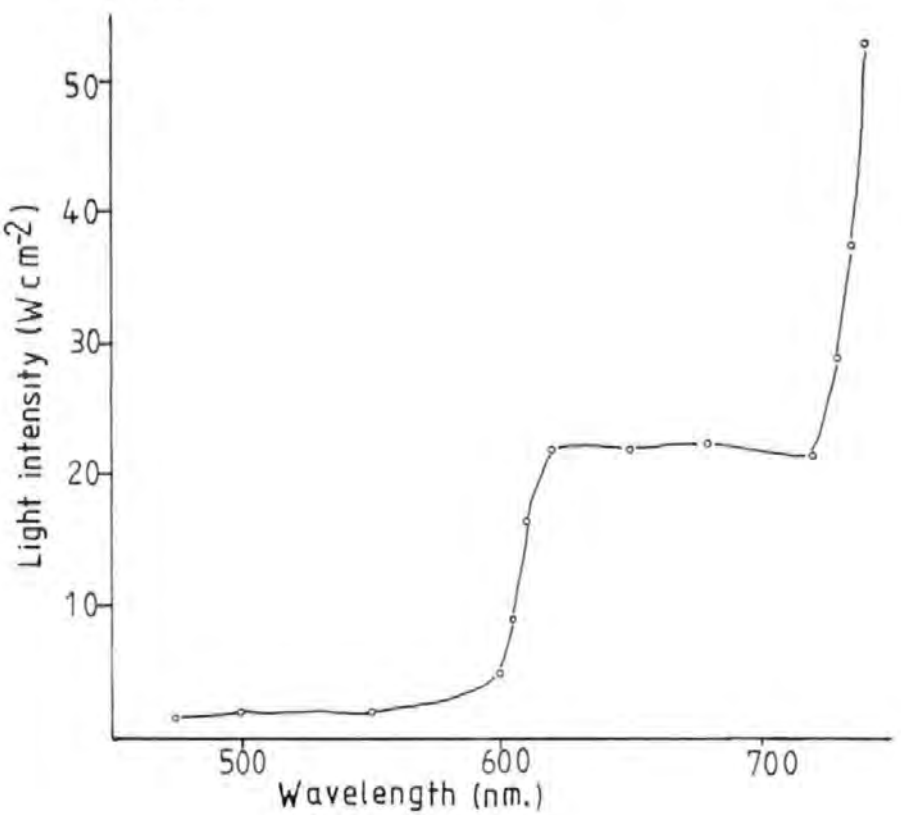

light
filter
black cover
water
perspex tank
petri-dish

FIG. 7.2


Light intensity (W c m-2)
50
40
30
20
10
500
600
700
Wavelength (nm.)

Statistical treatments

Most data were in the form of percentage germination figures. Any statistical treatment involving percentages was carried out on the values transformed by the arc-sine method as described by Bishop (1966). The transformed value was θ , in degrees, where $\sin\theta = \sqrt{(x/100)}$, in which x is the percentage to be transformed.

To measure the significance of differences between treatments, a t-test was carried out on pairs of mean treatment values. Calculations were reduced by producing a figure for the L.S.D. between mean values from the data presented in the relevant analysis of variance table.

SECTION 7.1

Effects of far-red light

Evidence was obtained from several different experiments that naturally buried seeds respond differently to a temperature and light regime from harvested seeds (see Chapters 3 and 5). When the results of tests on the thermogradient bars were compared with those of other workers using harvested seeds (Thompson 1977, Thompson and Grime 1983) it seemed that naturally buried seeds had an additional fluctuating temperature requirement which was not always found in harvested seeds. It has already been shown that burial can induce a light requirement in normally light-irresponsive seeds (Wesson and Wareing 1969(b)) and this may partly be due to the effects of far-red light filtered through leaves as seeds lie under vegetation before burial (Gorski 1975, Fenner 1980 and Silvertown 1980). It is possible that the fluctuating temperature requirement in naturally buried seeds may

also be induced in this way.

Phytochrome

Before describing the experiments in detail we must first briefly review the pathway of phytochrome photoconversion which underlies the observed seed responses. More detailed accounts of the photochemistry of the phytochrome pigment can be found in Mitrakos and Shropshire (1972) and Kendrick and Spruit (1977).

The pigment system called phytochrome behaves as follows; in darkness it is in a form which cannot release the seeds from dormancy. This pigment absorbs red light (it is therefore designated as Pr) and is quickly converted into a form which is active in the termination of dormancy but which absorbs far-red light (hence called Pfr). Absorption of far-red by Pfr causes its rapid reversion to Pr, dormancy is not broken, and no germination occurs. The light spectrum to which seeds the were exposed in these experiments (Fig.7.2) would be partially absorbed by both Pr and Pfr so an equilibrium will be established, designated as Pfr/Ptotal. By means of this photoequilibrium a seed is able to detect the light quality of its environment and seeds with a requirement for a relatively high Pfr/Ptotal ratio will not germinate in light filtered through green leaves which is rich in far-red wavelengths.

It is likely that a certain minimum concentration of Pfr molecules is needed to break dormancy i.e. a threshold concentration of Pfr must be reached before germination is stimulated. A population might include members with different thresholds and so as a whole it whould tend to show a graded response.

The photoconversions Pr$\rightleftharpoons$Pfr occur in more than one step in each direction. Several intermediates have been detected spectrophotometrically in plant tissues and of these, photoproducts are designated

by the prefix 'lumi' and products of dark reactions (relaxations) are given the prefix 'meta'. Further, the products of Pr are identified by R and those of Pfr conversion by F. The transformations are not understood in detail but an outline is given in Fig.7.3.

Effects of wetting and drying on phytochrome

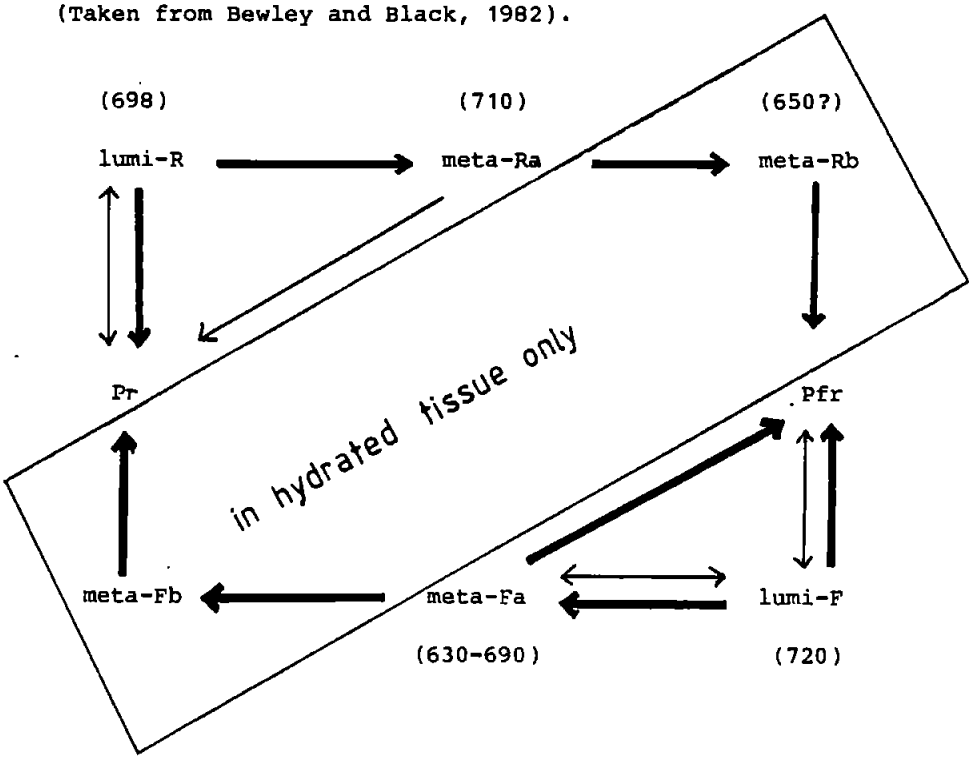
It can be seen from Fig.7.3 that the conversion of Pr to Pfr is prevented in dehydrated tissue. This is why light-requiring seeds generally are fully sensitive to light only when in the imbibed condition. Sensitivity to both red and far-red light is absent in the dry seed, i.e. about 6% moisture content, but it increases gradually to a maximum at water contents of between 13% and 22% (Hsiao and Vidaver 1971). Phytochrome has been measured in dry seeds of several species and a particular ratio of Pr to Pfr found. However during water uptake the concentration of phytochrome increases, the proportion of each form may change and, moreover, the difference spectrum of the new phytochrome may not always be the same as that of the original pigment (Spruit and Mancinelli 1969). These observations have been found to be due largely to hydration of previously existing intermediate forms of Pr or Pfr.

FIG. 7.3

Transformations of phytochrome.

Thin arrows are phototransformations and thick arrows are dark (thermal) transformations. Figures in parentheses are difference spectrum maxima.

(Taken from Bewley and Black, 1982).



When dry seeds are reimbibed in darkness Pfr may appear from intermediates such as meta-Rb which were trapped en route to Pfr during dehydration of the maturing seed or those (e.g. meta-Fa) which were formed while the seed was in the dry state. Intermediates formed by the action of far-red light during the drying process (e.g. meta-Fa) pass through meta-Fb to Pr on reimbibition. It follows from this that as seeds become imbibed in darkness the concentrations of Pr and Pfr gradually increase as the intermediate products are converted. The time scale of this process seems to vary from species to species and is dependent on temperature. In lettuce, full sensitivity to red light can be reached after exposure to water for about 100 minutes (Berrie, Paterson and West 1974) but Amaranthus retroflexus requires 12 hours imbibition at 20°C for complete hydration of phytochrome (Taylorson and Hendricks 1972a).

The hydration times quoted above suggest that 24 hours dark imbibition before exposing the seeds to light should be ample for phytochrome rehydration but evidence suggests that in some species (e.g. Amaranthus retroflexus) other processes which occur with imbibition are needed for the full expression of a light stimulus. Duke, Egley and Reger (1977) suggest that there may be gradual changes in the quantity or receptivity of a factor, X, with which phytochrome interacts and that in Rumex crispus for example, these changes are complete after 48 hours of dark imbibition at 15°C.

The effect of dark imbibition before irradiation is further complicated by the gradual reversion of Pfr to Pr in darkness, which is temperature dependent. Duke et al. (1977) suggest that dark reversion of Pfr in Rumex crispus seeds may be substantially complete after six days of dark incubation.

Experimental methods

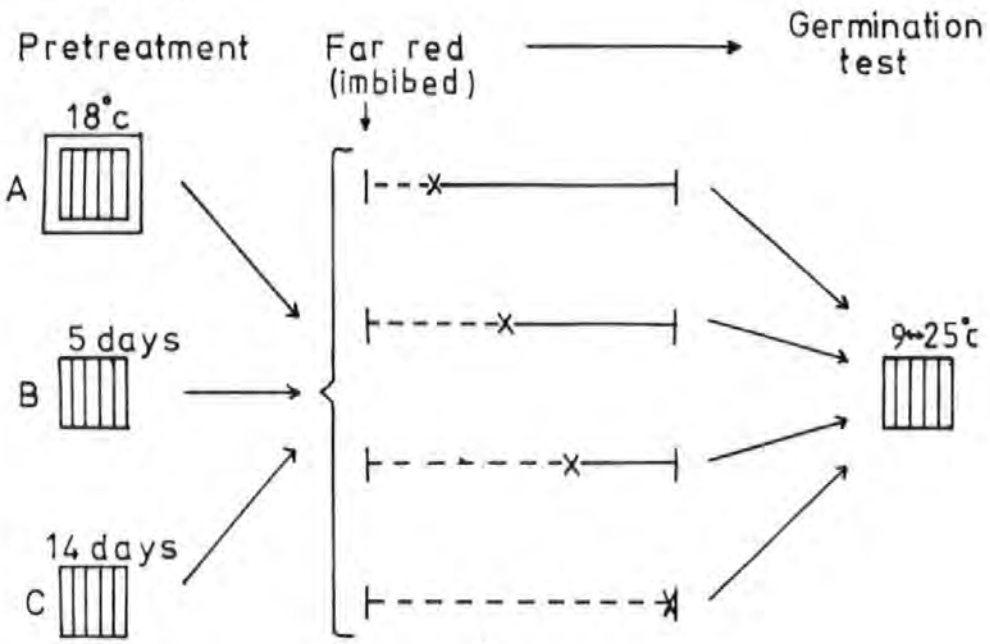
In an initial experiment to study the effects of increasing periods of the far-red rich spectrum on the subsequent response of Rumex obtusifolius seeds to fluctuating temperatures the apparatus shown in Fig.7.1 was used and seeds were then germinated in darkness at 9-25°C. As many of the responses described above are temperature dependent, batches of seeds receiving different light treatments were kept beneath the light box (22°C) for equal lengths of time before being transferred to the germination test conditions (see Fig.7.4).In order to expose the seeds to different periods of far-red light the petri dishes were covered by a double layer of foil after the required exposure time. To investigate the rate of dark reversion of Pfr in this species seeds were either exposed to far-red at the start of imbibition or imbibed in darkness for 5 or 14 days before exposure.

In a second experiment seeds were imbibed under the light box (Fig.7.1) for a total of 110 hours, without previous imbition. During this time the petri dishes were divided into three and each third covered in foil either initially, after 48 hours or after 110 hours. Thus some seeds received no far-red, others received 48 hours and the rest 110 hours, although all experienced the same temperature regime and were imbibed for the same length of time. The dishes were transferred to a growth cabinet (9/25°c) where subsamples received different numbers of consecutive diurnal temperature fluctuations before being transferred to 18°c for the remainder of the germination test.

FIG. 7.4.

Diagram to show the experimental method used to study the effect of various periods of far-red light on the subsequent response of Rumex obtusifolius seeds to fluctuating temperatures (see page 164 for details).

FIG. 7.4


Pretreatment
Far red
(imbibed)
Germination
test
18°c
A
5 days
B
14 days
C
9↔25°c

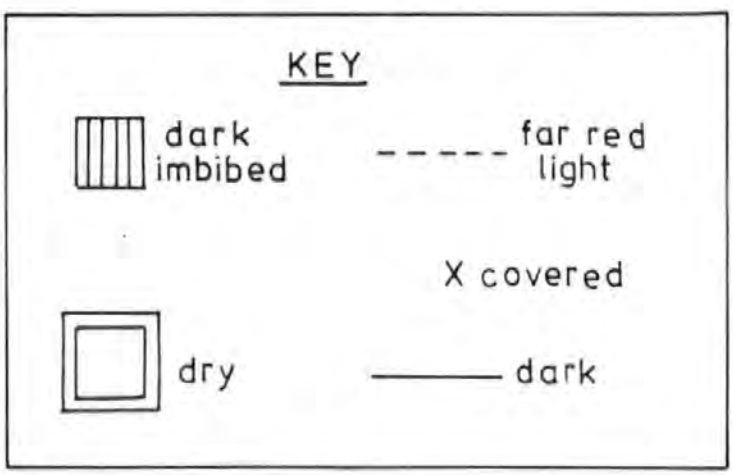

KEY
dark
imbibed
far red
light
X covered
dry
dark

FIG. 7.5.

The results of the experiment shown in Fig. 7.4.

The mean germination percentage of batches of seeds having received Pretreatment A (●); Pretreatment B (○); or Pretreatment C (X); before being exposed to far-red light. The germination test was carried out in darkness in a fluctuating temperature regime (9/25°c).

FIG. 7.5

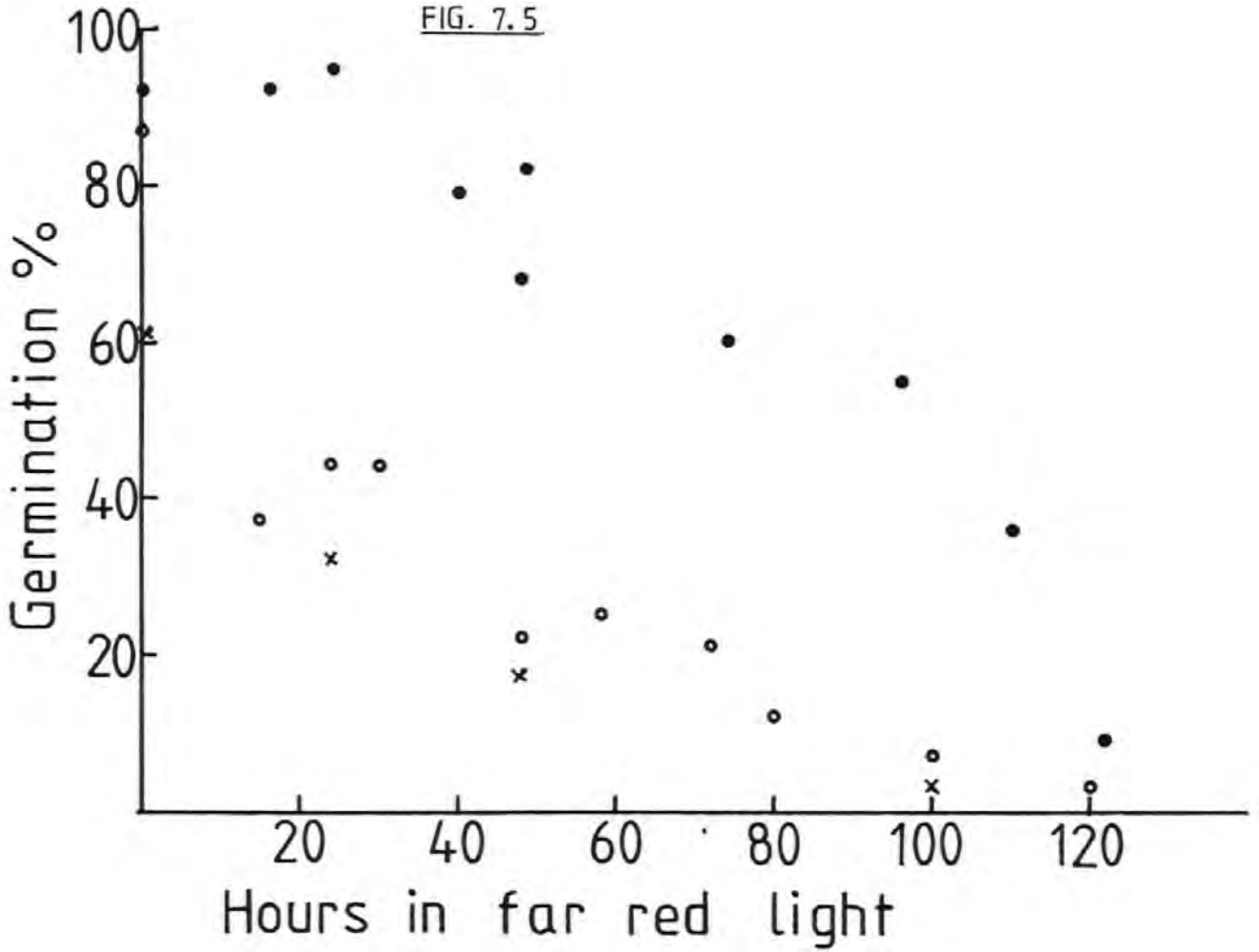
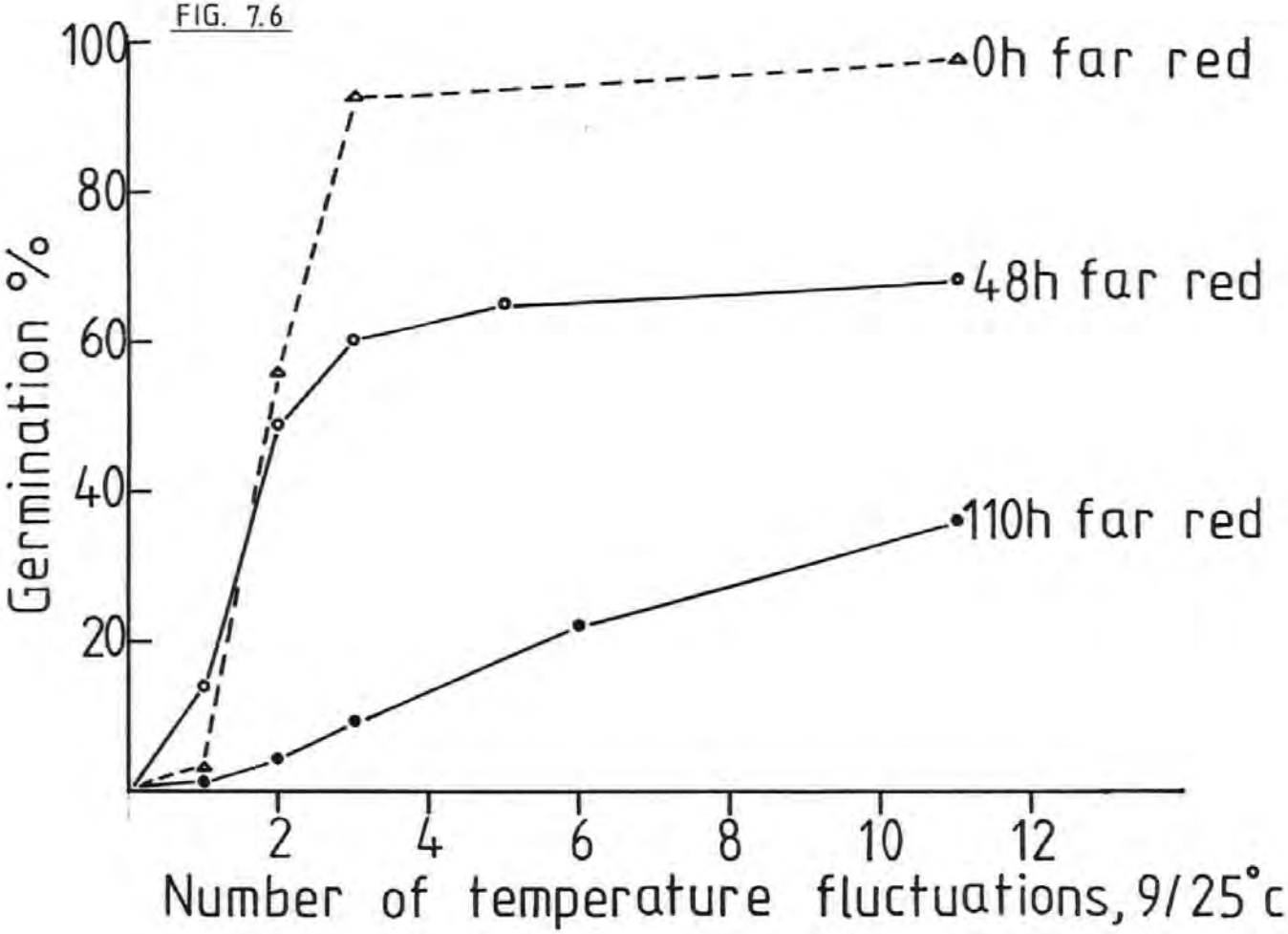

100
80
60
40
20
Germination %
20
40
60
80
100
120
Hours in far red light

FIG. 7.6.

Results of an experiment to show how various periods of exposure to far-red light affect the subsequent germination response of Rumex obtusifolius to increasing numbers of consecutive diurnal temperature fluctuations (9/25°c).

(Details of experimental method, Page 164).

FIG. 7.6


100
80
60
40
20
Germination %
0h far red
48h far red
110h far red
2
4
6
8
10
12
Number of temperature fluctuations, 9/25°c

RESULTS AND DISCUSSION

The results from these experiments (Figs. 7.5 and 7.6) show an obvious effect of far-red light on subsequent seed germination whether or not the seeds have previously been imbibed in darkness before the light treatment. The longer the exposure to far-red light the lower was the subsequent germination percentage in a fluctuating temperature regime in darkness. When seeds were imbibed in the dark (18°C) for five or fourteen days before the far-red treatment the germination percentage was significantly lower for similar periods of exposure. There was no significant difference in response to various periods of far-red treatment between seeds previously imbibed in the dark for five days or fourteen days. There was very little germination (<10%) after 120 hours of far-red light whether the seeds had previously been imbibed in darkness or not. It is likely that several different processes are occurring in the seeds at different rates, some of which involve the pigment phytochrome. We will therefore look at the evidence which the results provide for each of these processes.

Effect of hydration

If far-red light can only act on hydrated Pfr, and some intermediate forms of phytochrome revert to Pfr on hydration, one would expect there to be a rapid response to far-red light when seeds had been imbibed in the dark for several days but a much more gradual response when seeds start imbibition under far-red light. This was in fact the case, as the germination of seeds previously imbibed in darkness for 14 days was reduced to 61% after only 20 minutes far-red whereas those imbibed under the lights required 60-80 hours exposure to be inhibited to the same extent. The time taken for the hydration of the phytochrome system varies between species (Bewley and

Black 1982) but is unlikely to exceed 24 hours. The additional time required for the seeds to be fully responsive to far-red could be explained by the suggestion of Duke, Egley and Reger (1977) that there may be gradual changes as hydration proceeds in the quantity or receptivity of a factor, X, with which phytochrome interacts.

Low energy reaction of phytochrome

Imbibed, non-dormant seeds of several species are inhibited by exposure to far-red light for a few minutes or hours, e.g. Oryzopsis miliacea, (Negbi and Koller 1964) through phytochrome operating in the low-energy mode. This mechanism which causes a simple reversal of Pfr to Pr would account for the initial drop in germination of Rumex obtusifolius to approximately 60% but not for the continued drop in germinability with up to 120 hours exposure to far-red.

Effects of prolonged far-red light

Unlike the rapid reversal of Pfr to Pr described above, some effects of far-red are both time- and irradiance-dependent. Prolonged irradiation with far-red light can prevent germination when it is given just before the radicle is about to emerge, which suggests that the high-irradiance reaction (HIR) of phytochrome is involved (Smith 1974). The mechanism behind this reaction is unclear but it is known that both the Pfr/Ptotal ratio and the rate of phytochrome conversions (i.e. $Pr \rightleftharpoons Pfr$) can be important. Taylorson (1972) suggests that the HIR functions by a continuos displacement of Pfr from its site of action.

The results shown in Fig.7.5 suggest that Rumex obtusifolius seeds can be progressively inhibited by periods of far-red light of up to five days even after the dark imbibition treatment. This points to

the involvement of the HIR but there is an alternative explanation which is mentioned below.

Non-photochemical reversion of Pfr

In addition to the phenomena discussed above, another important occurrence infuencing the state of the photoreceptor is the thermal reversion of Pfr to Pr in seeds imbibed in darkness. Frankland (in Smith 1981) uses this phenomenon to explain the failure of seeds to germinate in response to prolonged irradiation at low fluence rate even when the Pfr/Ptotal ratio calculated on the basis of the 660/730nm ratio is still fairly high (which is the case in the experiments conducted here). He suggests that at very low fluence rates the rate of non-photochemical reversion of Pfr to Pr will be greater than the rate of photochemical conversion of Pr to Pfr. However, if the dark reversion of Pfr was important in Rumex obtusifolius one would expect that seeds imbibed in darkness for 5 days before being transferred to fluctuating temperatures would be less germinable than those without the dark imbibition period. This is clearly not the case (Fig.7.5).

Effect of fluctuating temperatures

The seeds in initial experiments (Fig.7.4) were germinated in a diurnally fluctuating temperature regime (10 hours at 25°C and 14 hours at 9°C) and subsamples from the second experiment were given increasing numbers of consecutive diurnal fluctuations before being germinated at a constant temperature (18°C) in darkness. The results (Fig.7.6) show that the final germination percentage was clearly reduced by the far-red treatment as 98% germinated after no light treatment, 68% after 48 hours and 36% after 110 hours far-red when

tested in continous fluctuations. There also appears to be an interaction effect between the length of exposure to far-red and the subsequent temperature regime. The longer the exposure to far-red the greater was the number of fluctuating temperature cycles required to achieve the final total number of germinating seeds. In the 110 hour far-red treatment, out of the seeds with the potential to germinate only half would do so after five consecutive days of the fluctuating temperature regime. In contrast, nearly all the seeds with the potential to germinate after the other two treatments would do so after five diurnal fluctuations.

Seed dormancy of many species is broken in darkness by alternating temperatures or temperature shifts, and in many cases it can be shown that their effectiveness depends upon the presence of Pfr (though small amounts are adequate) already within the seed (Taylorson and Hendricks 1972). The results described above are consistent with this view. Seeds exposed to increasing periods of far-red would be expected to have less Pfr when transferred to fluctuating temperatures and the temperature regime would therefore be less effective in promoting germination.

There is no clear experimental evidence for the mechanism behind these Pfr/ temperature interactions but Van der Woude and Toole (1980) argue that there is a strong indication that Pfr forms some kind of association with a cell membrane or membranes, which is facilitated when the membrane has undergone a temperature-induced transition. Temperature fluctuations may therefore be involved in changing the membrane structure so that Pfr can be more easily incorporated in to the matrix.

Ecological Implications

These results suggest that the moisture conditions under the canopy are important in governing a seed's response to far-red light after shedding from the mother-plant. A seed which has been imbibed in the dark for several days before receiving a far-red stimulus is able to respond much more rapidly than one which has previously been dry. Little is known about the length of time for which seeds shed in field situations are exposed to far-red light before burial. This would depend on the thickness of the canopy, the amount of litter and the rate of burial.

The response of an individual seed to far-red light is likely to depend on its moisture content. Fenner (1980) found that a light requirement could be induced in dry Bidens pilosa seeds placed under a natural leaf canopy for 12 days but to a lesser degree than imbibed seeds. Buried seeds, unless very near the soil surface or during lengthy spells of dry weather, are generally continually imbibed. The results (Fig.7.5) suggest that if such a buried seed is unearthed beneath a canopy it is rapidly inhibited by far-red light and will not germinate. The inhibition by canopy light may be less rapid when dry mature seeds from the mother plant are first shed and then become imbibed beneath a canopy. Inhibition of germination by canopy light would be less important for survival in this case as many species show innate dormancy when they are first shed.

The results shown in Fig.7.6 suggest that seeds receiving longer periods of far-red light before burial would subsequently be less responsive to short periods of temperature fluctuations. However, in these experiments the interaction between far-red and one particular amplitude of temperature fluctuation (16°C) was investigated and it is likely that different amplitudes would affect the response to far-red

to varying degrees. This would be a useful area for future studies as it is possible that one factor governing the lack of germination of naturally buried Rumex obtusifolius at small temperature fluctuations (Fig.5.7) is their previous exposure to far-red light. This may also be the case in an experiment described in Chapter 5 in which large flushes of Rumex obtusifolius and Holcus lanatus emerged after five or more consecutive days of fluctuations greater than 5°C (Fig. 5.11-5.13).

These observations prompted an investigation into how many consecutive fluctuations of a certain amplitude were needed to break dormancy in Rumex obtusifolius seeds and also if the effects of individual large fluctuations could be additive over a period of time, particularly when buried seeds near the soil surface may dry out between temperature fluctuations. The results of some experiments to investigate these questions are described in the next section of this chapter.

SECTION 7.2

Effects of wetting and drying cycles

Field observations have shown that the surface layer of soil is likely to dry out significantly in late spring and summer even under a canopy of vegetation (Table 7.1) and this affects the number of seedlings emerging (Fig.4.10). This agrees with suggestions that rainfall is important in governing flushes of germination in the field (Benjamin 1974, Roberts and Potter 1980). Wetting and drying cycles in the field may affect a seed°s response to the dormancy breaking stimulus of fluctuating temperatures. This was investigated for naturally buried seeds in soil on the thermogradient bars and for harvested Rumex obtusifolius seeds.

TABLE 7.1

Percentage soil moisture at two depths on 23-6-83.

	DEPTH	
	SOIL SURFACE	5cm BELOW
Site 2 (Derelict pasture)	16.1	13.6
Site 3 (Daffodil field)	3.2	13.6
Site 4 (Potato field)	3.3	11.8

Methods using naturally buried seeds

Soil collected from the derelict pasture (Plate 4.1) on 4-11-82 was prepared in the normal way (described in Chapter 2) and two subsamples were tested in darkness on the thermogradient bars. The first subsample was wetted, given three days of the fluctuating temperature regime (see Fig.2.4 and 2.5) then left to dry out completely for 24 hours before being rewet under a constant temperature regime of 18°C. The second subsample was wetted and received the fluctuating temperature regime continually. The number of seedlings emerging after fourteen days was counted. The method of expressing the seedling data is described in detail in Chapter 2.

RESULTS

The results (fig.7.7) from the two soil subsamples were very similar. There was little difference between the pattern of seedling emergence and numbers of seedlings after three days fluctuations or after fourteen days fluctuations. The dormancy breaking stimulus of three days fluctuating temperatures was therefore approximately equal to that of fourteen days. The stimulus was retained by the seeds during the 24 hour dry period and then manifested at the constant temperature.

More controlled experiments were then carried out using harvested Rumex obtusifolius to find the minimum number of consecutive diurnal temperature fluctuations needed to break dormancy in seeds imbibed in the dark and to investigate the effect of drying seeds between diurnal temperature fluctuations.

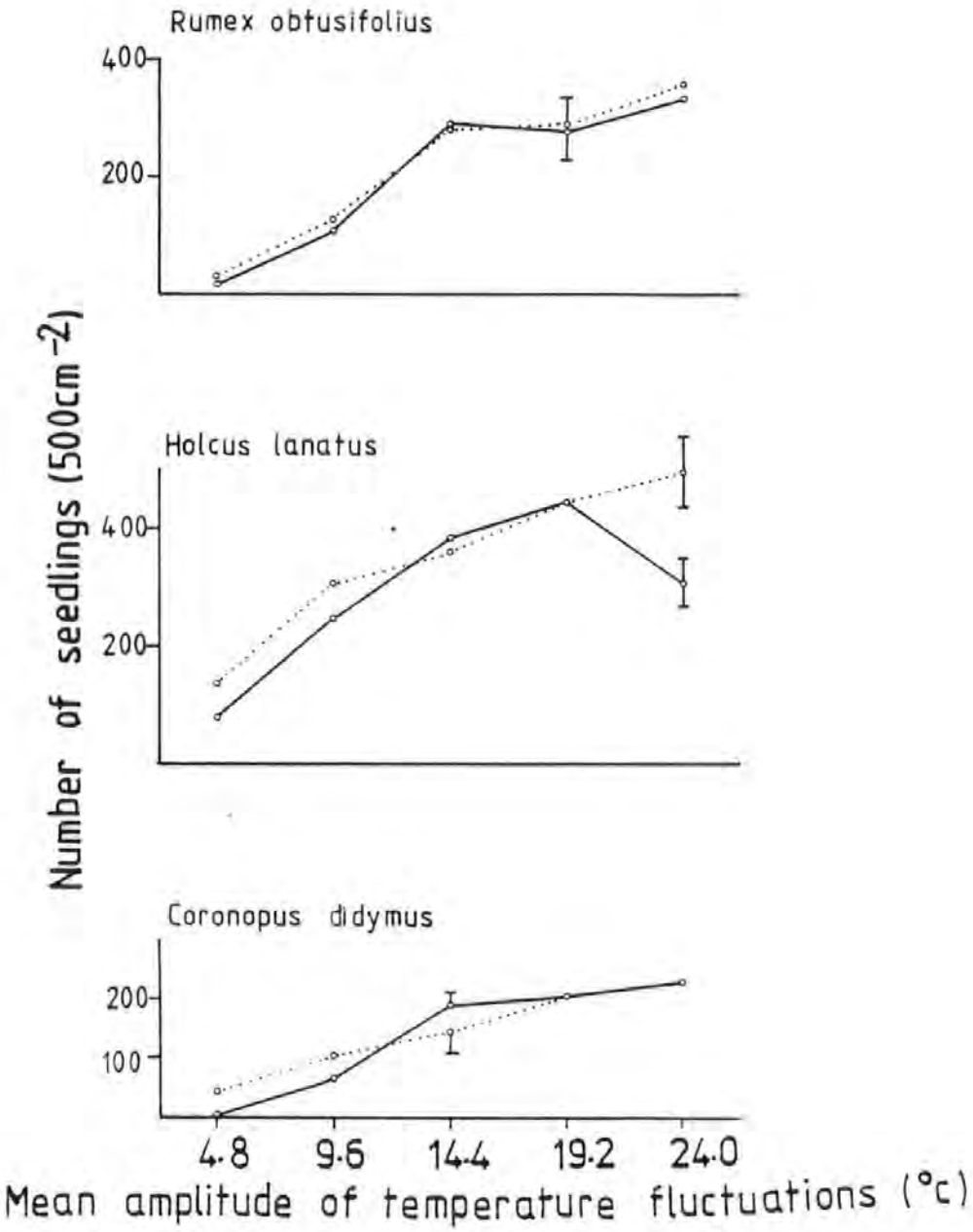
FIG. 7.7.

Numbers of seedlings emerging on the thermogradient bars in response to continuous temperature fluctuations (O——O) and to three consecutive diurnal fluctuations followed by drying and re-wetting at a constant temperature (18°c), (O·····O).

Soil was tested in a fourteen hour photo-period.

Bars indicate L.S.D. (P = 0.05).

FIG. 7.7


Rumex obtusifolius
400
200
Holcus lanatus
400
200
Coronopus didymus
200
100
Number of seedlings (500cm⁻²)
4·8
9·6
14·4
19·2
24·0
Mean amplitude of temperature fluctuations (°c)

Methods using harvested Rumex obtusifolius seeds

The methods used to prepare and test Rumex obtusifolius are described at the beginning of this chapter. For these experiments seeds were imbibed in darkness for different numbers of consecutive diurnal temperature fluctuations then transferred to a constant temperature (18°C) and the number of germinations counted after a further 14 days. Other batches of seeds were given a single temperature fluctuation cycle (24 hours) then opened in the dark to dry during the following cycle. They were then reimbibed at the constant temperature or for a further temperature cycle. The wetting and drying procedure was repeated several times for some subsamples before they were finally left to germinate at 18°C. In practice the seeds were completely dry for 12-15 hours before being reimbibed during the following temperature cycle. This technique simulated field conditions in which seeds near the soil surface (though still in darkness) dried out frequently between experiencing temperature changes. Field observations at the derelict pasture site suggest that this would be a common occurrence when the soil begins to dry out at the end of spring. Seeds may imbibe some dew but then rapidly dry out as temperatures become hotter towards midday.

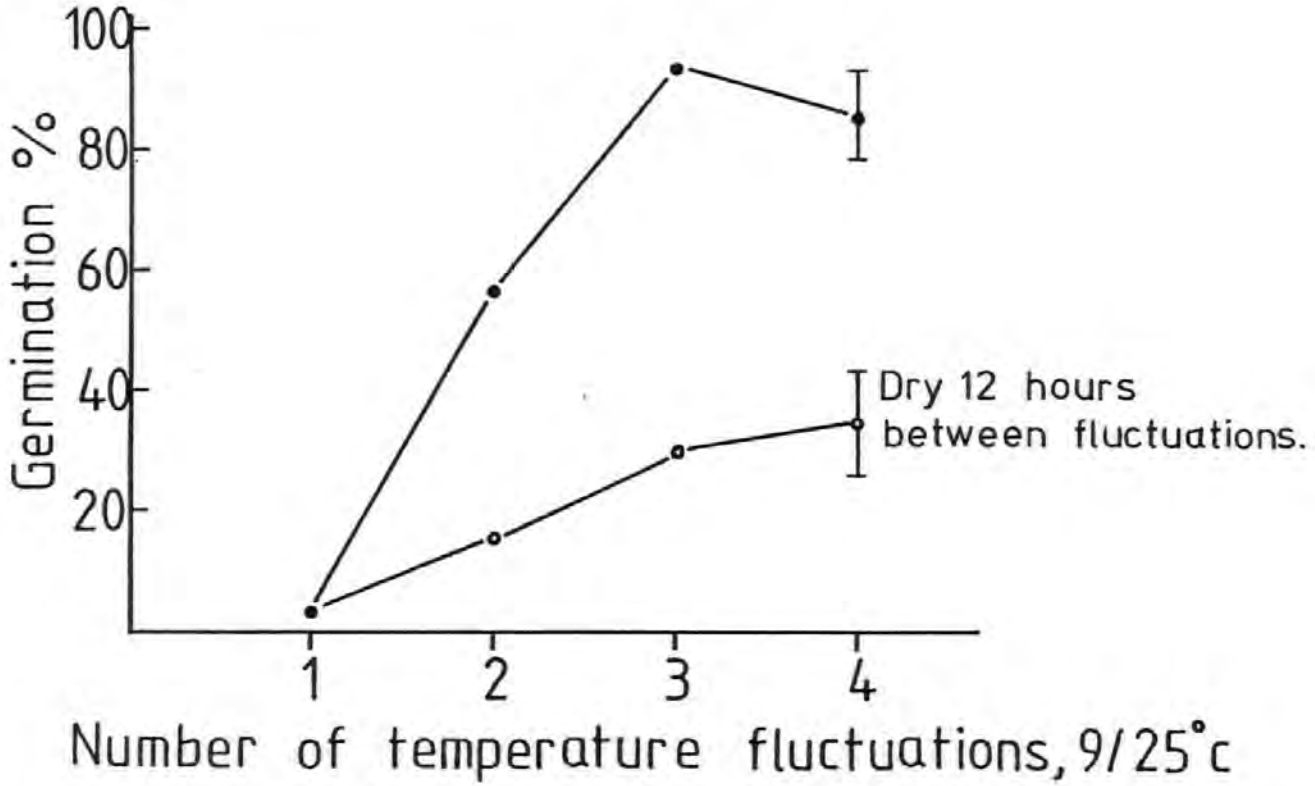
FIG. 7.8.

Germination response of Rumex obtusifolius to various numbers of consecutive diurnal temperature fluctuations (9/25°c) before being transferred to a constant temperature (18°c).

See Page 177 for experimental method.

Bars indicate L.S.D. (P = 0.05).

FIG.7.8 Seeds kept in continuous darkness.



RESULTS

The results are shown in Fig.7.8. The continuously imbibed seeds required at least three consecutive days of temperature fluctuations for all the seeds in the population with the potential to respond to the stimulus to do so. This was shown by their subsequent ability to germinate when transferred to the constant temperature (18°C). Very few seeds were able to germinate after a single temperature fluctuation but nearly 60% were able to germinate after two consecutive days of fluctuations. The seeds that were dried between single cycles of temperature fluctuations were less likely to germinate when subsequently tested at a constant temperature. There was some additive effect of increasing numbers of fluctuations with drying between each one but this was small compared with continually imbibed seeds. After four fluctuations only 35% of the seeds were stimulated to germinate.

Discussion annd Ecological Implications

The above observations again underline the heterogeneity found in the germination response to different stimuli in seed populations. There was a big difference between the number of seeds germinatin g after either two or three temperature fluctuations when imbibed continuously and this diversity in response would affect the timing of buried seed germination in the field.

Buried seeds in a field site are found in many different microsites which would dry out at different rates depending on factors such as vegetation cover, soil type, soil drainage and litter cover. The drying of seeds between temperature fluctuations seems to affect the degree of dormancy breaking by this stimulus (Fig.7.8) and the results suggest that seeds in more exposed microsites would be less

likely to germinate after a series of normally stimulatory temperature fluctuations because they dry out more rapidly. A lack of response to fluctuations at exposed sites could be a useful drought avoiding mechanism.

Further work needs to be carried out to investigate the range of seed moisture contents for different species above which they can respond to a temperature shift. There may also be distinct differences between species in the degree to which they have to dry out in order to provide a 'break' between fluctuations. Factors such as these which govern the germination requirements of species may be important in determining their regeneration niche in the field (see Chapter 6 for discussion of regeneration niche).

It has already been shown that differential germination responses to levels of soil moisture can contribute to the coexistence of some species. Three species of Ranunculus coexist in ridge and furrow grassland on the basis of moisture microhabitat differentiation by the seeds rather than by the adult plants (Harper and Sagar 1953). Harper, Williams and Sagar (1965) have also shown that different soil surface features favoured germination by a variety of different species, the size and shape of different microsites corresponding to those of various seeds. It was subsequently found that imbibition and sustained hydration were promoted in suitable microsites (Harper and Benton 1966) which would increase the dormancy breaking effect of periods of large temperature fluctuations. However, Pickett and Bazzaz (1978) give an example of six co-occurring annual species which have very similar soil moisture requirements for germination and they suggest that differentiation in this aspect of the regeneration niche is unnecessary due to the pattern of soil moisture changes in time and space and differences between the species in other specialized cues

for breaking dormancy.

The results shown in Fig.7.7 suggest that the dormancy breaking effect of three consecutive cycles of fluctuating temperatures can be fully retained during a dry period of at least 24 hours. In a field situation, particularly if the stimulus can be retained for a drought of longer than 24 hours, this would account for a flush of seedlings from buried seeds appearing immediately after rainfall in the summer (Roberts and Potter 1980). The experiments of Vincent and Cavers (1978) on the effects of wetting and drying on harvested Rumex crispus suggest that seeds at the soil surface exposed to alternating temperatures and light would become progressively more responsive to remoistening during successive wet periods with the result that seeds which did not germinate before drying would germinate very quickly the next time the soil became moist.

The different responses to far-red light and temperature fluctuations discussed above in Sections 7.1 and 7.2 may help to account for some of the heterogeneity found in the germination responses of naturally buried seeds to the temperature regime on the thermogradient bars (Chapter 5). It is possible that seeds having received longer periods of far-red light or fewer temperature fluctuations when imbibed before burial would subsequently be less responsive to the fluctuating temperature regime when tested in this way.

The subsequent effectivenss of an initial dormancy inducing or alleviating stimulus experienced while the seed is on the parent plant or lying under the leaf canopy must depend on how long the effect of the stimulus is retained by the seed. If the effects of far-red light and temperature fluctuations deteriorate after a short period of

burial then other factors would be more important in governing the germination responses of buried seeds on the thermogradient bars. Experiments designed to investigate the ability of seeds to 'remember' various initial stimuli when stored (equivalent to burial) under different conditions are discussed in the following section.

SECTION 7.3

Preconditioning - retention of various initial stimuli by seeds

It is known that the subsequent germination requirements of seeds can be affected by conditions experienced during maturation on the mother-plant. For example, germination can be reduced by increasing the temperature or reducing the photoperiod (Grantlipp and Ballard 1963) in which the mother-plant grew and this effect can last for long periods (Gutterman 1973).

Individual seeds in a naturally buried seed population have received many different stimuli from the time of maturation to shedding and burial. If the residual effects of these stimuli are retained for long periods this would help to account for their heterogeneity in response to a given stimulus such as fluctuating temperatures. Controlled experiments were therefore carried out on harvested Rumex obtusifolius seeds to examine this hypothesis.

METHODS

Imbibed seeds were subjected to various initial treatments for a short time (32 hours) before being dried and stored at 18°C in darkness. These treatments simulated different environmental stimuli that seeds in a particular population may have experienced before or during burial before being dried in a drought.

Subsamples of the seeds were tested to assess their retention of the effects of the stimuli after increasing periods of dry storage. The first set of germination tests involved reimbibing the seeds in darkness at 18°C and recording germination after 14 days (Fig.7.9). This simulated conditions in the field after rainfall for seeds that were either quite deeply buried or were near the soil surface but beneath thick vegetation and/or litter which would greatly reduce the temperature fluctuations in the soil.

The various initial treatments or pretreatments mentioned above are represented diagramatically in Fig 7.9. Pretreatment A simulated seeds which were in an environment where they received no light stimulus between being shed from maternal seed coverings and being buried. Pretreatment B would be similar to that experienced by seeds which were shed on a bare soil surface and received unfiltered light before being buried by cultivation or earthworms. Pretreatment C would be experienced by seeds buried near the surface of bare soil before it was covered by vegetation and Pretreatment D would be experienced by most seeds shed in grassland or in an actively growing crop where the light would be filtered by the green leaves and the proportion of far-red light thereby increased before it reached seeds lying on the soil surface.

FIG. 7.9.

Diagram to show the experimental method used to investigate the retention of various initial preconditioning treatments by Rumex obtusifolius during dry storage.

Details see page 182.

FIG. 7.9

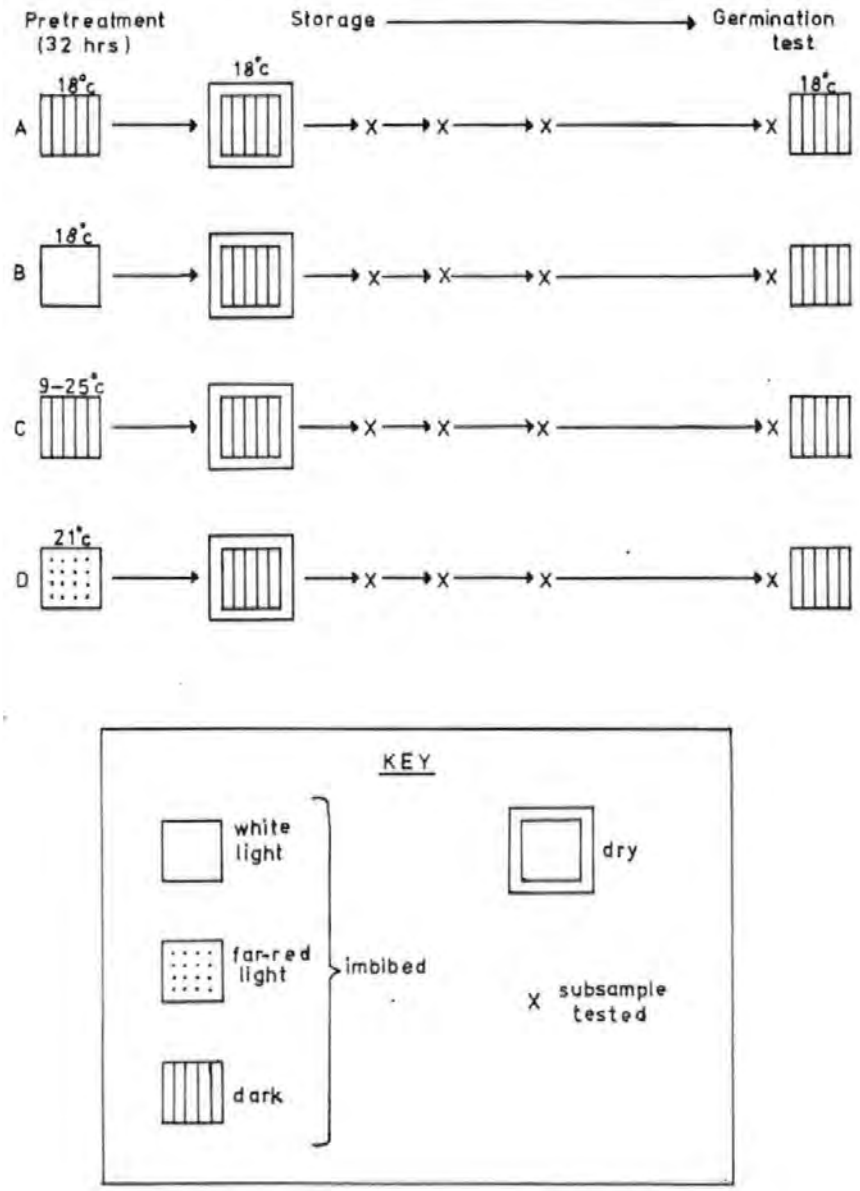

Pretreatment
(32 hrs)
Storage
Germination
test
18°c
18°c
18°c
A
18°c
B
9–25°c
C
21°c
D
X
KEY
white
light
far-red
light
dark
imbibed
dry
X subsample
tested

TABLE 7.2

Germination of Rumex obtusifolius in continuous darkness at 18°c after different pretreatments and periods of dry storage.

DRY STORAGE (days)	PRETREATMENTS			
	A (18°c,dark)	B (18°c,light)	C (9/25°c,dark)	D (22°c,far-red)
2	1	33	13	2
4	1	24	13	0
7	1	20	12	1
24	1	22	14	0

RESULTS AND DISCUSSION

The results are shown in Table 7.2 from which it is clear that the preconditioning treatments had a significant effect on the subsequent germination percentage.

Very few seeds that had previously received far-red light or no light stimulus (Pretreatments A and D) germinated in darkness at the constant temperature(18°c). Those that had been exposed to the fluctuating temperatures previously (Pretreatment C) were a little more germinable (12-14%) and up to a third of those having received white light (Pretreatment B) could subsequently germinate in darkness at 18°c. Increasing the length of dry storage after the pretreatment up to 24 days did not appear to affect the seeds subsequent response to that pretreatment. These results suggest that the past history of individual seeds in the seed bank can be important in determining whether they will germinate under a particular set of conditions.

Seeds at regularly cultivated sites such as agricultural fields may be buried after lying on fallow ground and the results from Pretreatment B suggest that a significant proportion may subsequently germinate deep in the soil and then probably die by exhausting their seed reserves before reaching the soil surface.This has been cited as a possible cause for exhaustion of the seed bank by several previous workers (e.g. Roberts and Dawkins 1967). However, in most natural and semi-natural habitats where there is a more or less continuous plant cover seeds are likely to lie beneath vegetation before burial and thus be exposed to far-red light which would prevent germination at unfavourable microsites characterised by constant temperatures.

The retention of a previous light stimulus during long periods of drought would account for the rapid flushes of seedlings observed after summer rainfall (Roberts and Potter 1980).

Effect of preconditioning on germination response to a brief white light stimulus

The ungerminated seeds from the previous experiment were kept imbibed in the dark (18° c) for a further 6 days (i.e. 20 days in total) before being exposed to five minutes white light in the incubator and returned to darkness (18° c).

This treatment simulated field conditions in which seeds that had been deeply buried for some time were then exposed briefly to light at the soil surface and reburied. This could be caused by ploughing or other soil cultivations. No additional germination beyond that shown in Table 7.2 took place in the six days before receiving the light stimulus. The total germination percentages fourteen days after the white light stimulus are shown in Table 7.3.

The increase in percentage germination (figures in brackets, Table 7.3) suggests that the different preconditioning treatments no longer affect the subsequent response to light after a period of dark imbibition. There was little response to the brief light stimulus after any treatment and seeds were completely irresponsive after being stored dry for 24 days before being reimbibed. These results should be compared with those of another similar experiment in which the seeds were only reimbibed in darkness for two days before being given the five minute white light stimulus (Table 7.4).

TABLE 7.3

Percentage germination in continuous darkness (18°c) after 20 days dark imbibition (18°c) and a brief exposure to white light (5 min.).

DRY STORAGE	PRETREATMENTS			
(days)	A	B	C	D
	(18°c,dark)	(18°c,light)	(9/25°c,dark)	22°c,far-red)
2	18(17a)	34(1b)	13(0b)	3(1b)
4	8(7a)	30(6a)	16(3a)	5(5a)
7	8(7a)	27(7a)	20(8a)	7(6a)
24	1(0a)	22(0a)	14(0a)	0(0a)

The percentage increase over the initial germination in darkness (Table 7.2) is shown in brackets.

Within a row, treatments with a subscript letter in common do not differ significantly at P = 0.05 using the student's t-test.

It is possible that the effects of the pretreatment would be retained during this short period of dark imbibition and affect the subsequent response to light. This treatment simulates field conditions in which seeds have only been buried in microsites with constant temperatures for a very short time before being exposed at the soil surface and reburied.

Table 7.4. Germination in continuous darkness (18°c) after a brief exposure to white light(5mins) after 2 days dark imbibition.

DRY STORAGE	PRETREATMENTS			
(days)	A	B	C	D
	(18°c,dark)	(18°c,light)	(9/25°c,dark)	(21°c,far-red)
2	46(45)^{a}	45(12)^{b}	83(70)^{c}	32(30)^{d}
4	53(52)^{a}	64(40)^{b}	91(78)^{c}	35(35)^{b}
7	55(54)^{a}	62(42)^{b}	88(76)^{c}	28(27)^{d}
24	36(35)^{a}	51(29)^{a,c}	84(70)^{b}	22(22)^{c}

Percentage increase over the germination in darkness (Table 7.2) is shown in brackets. Within a row, treatments with a superscript letter in common do not differ significantly at P = 0.05 using the student's t-test.

The results show that there are significant differences in germination response caused by the various pretreatments. The response to white light was very variable between seed batches from each pretreatment but this seems to be an indication of between seed heterogeneity rather than an effect of dry storage time as there is no clear correlation between storage time and total germination for any treatment. Those seeds that had been exposed to far-red light were the least responsive to a short period of white light, the increase in germination being approximately 30%. There was a relatively greater increase in germination in seeds not having previously received light compared with light pretreated seeds, but the greatest response to white light was shown by seeds previously exposed to fluctuating temperatures.

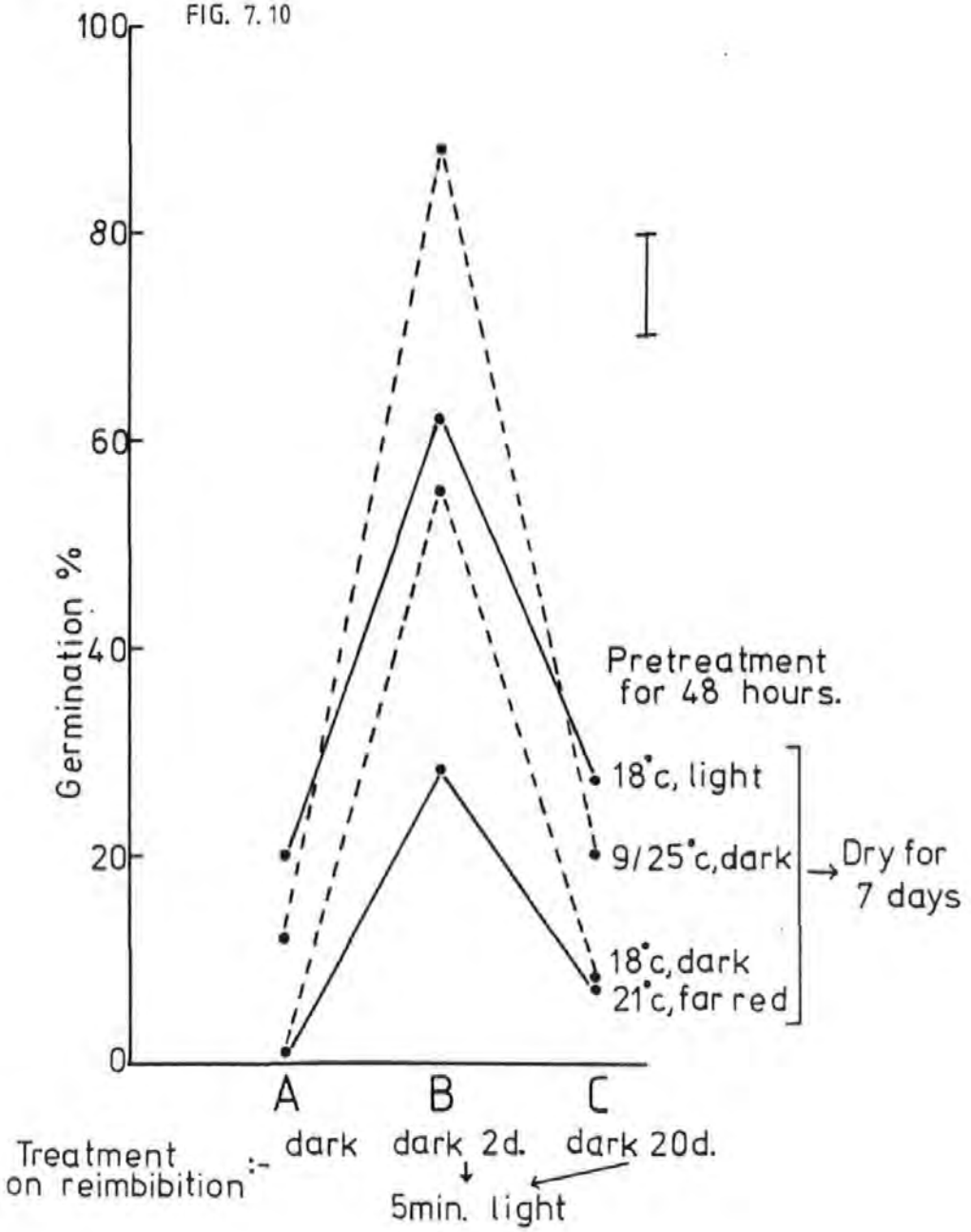
A positive interaction between light and alternating temperatures when given simultaneously has been found for several weed species (Roberts and Benjamin 1979, Vincent and Roberts 1977). These results (Table 7.4) suggest that this interaction can still take place when the seeds are exposed to a fluctuating temperature regime in darkness, dried for several weeks then experience light after being reimbibed. However the results in Table 7.3 show that this interaction would no longer be expected when the seeds have been imbibed in darkness for more than a few days.

FIG. 7.10.

Summary of the results shown in Tables 7.3 and 7.4 for pretreated Rumex obtusifolius seeds stored dry for 7 days before being reimbibed in darkness (18°c). Seeds were imbibed for 2 days (B); or 20 days (C); before receiving a brief white light stimulus (5 min).

Bar indicates L.S.D. (P = 0.05).

FIG. 7.10


100
80
60
40
20
0
Germination %
Pretreatment
for 48 hours.
18°c, light
9/25°c, dark
18°c, dark
21°c, far red
Dry for
7 days
A
B
C
Treatment
on reimbibition :-
dark
dark 2d.
5min. light
dark 20d.

The results of these two experiments for one storage period are summarised in Fig. 7.10 and suggest that recently buried seeds are much more likely to germinate in potentially adverse microsites (characterised by darkness and constant temperatures) than those seeds which have survived in the seed bank without germinating even for a few days. The lack of responsiveness to a brief light stimulus after a period of dark imbibition indicates that these seeds must be in a state of secondary dormancy. This type of dormancy is known as skotodormancy and is induced when light requiring seeds are held imbibed in darkness for several days. Skotodormancy is broken in Verbascum blattaria and Polygonum persicaria by chilling (Staniforth and Cavers 1979) and in lettuce by various combinations of light, gibberellin, cytokinin, thiourea and ethylene (Bewley 1980, Vidaver and Hsiao 1975). The possible underlying mechanism for this type of response is discussed at the end of this chapter.

The effects of skotodormancy on subsequent germination response.

In a separate experiment the effects of skotodormancy on the seeds' subsequent response to fluctuating temperatures in darkness were investigated. The pretreatments were given as before, the seeds dried for ten days in darkness and then reimbibed at 18°c in the dark. Germinated seeds were counted and removed after 20 days and the remaining seeds were either given five minutes light as above or transferred to a diurnal fluctuating temperature regime (9/25°c) in the dark. This simulated field conditions in which seeds that have been buried for some time become exposed to fluctuating temperatures without being exposed to light. This may happen when deeply buried seeds are brought nearer an exposed soil surface by the action of worms and other invertebrates or when the amplitude of temperature

fluctuations increases at a given depth due to the removal of above ground vegetation and/or litter. The results (Table 7.5) show the increase in percentage germination after the light or alternating temperature stimulus.

As before the results show that the effects of the initial pretreatments are lost during a period of dark imbibition. The seeds are much more responsive to fluctuating temperatures than to a brief light stimulus when in a state of skotodormancy. In the field situation seedlings produced from seeds experiencing fluctuating temperatures in darkness would be more likely to survive than those produced from seeds which receive light and then become deeply buried again. The responses described above would generally therefore, prevent seeds from germinating under unfavourable conditions for seedling survival.

TABLE 7.5

Increase in percentage germination after a brief exposure to white light (5min.) or to temperature fluctuations (9/25°c) after 20 days dark imbibition (18°c).

	PRETREATMENT			
	A (18°c,dark)	B (18°c,light)	C (9/25°c,dark)	D (22°c,far-red)
5 min. light	12a	3b	12a	7a,b.
Temperature Fluctuations	65a	52b	43c	44b,c.

Within a row, treatments with a subscript letter in common do not differ significantly at P = 0.05 using the student's t-test.

Ecological implications of preconditioning.

It seems likely that previous stimuli can continue to affect the germination responses of seeds for long periods when the seeds are buried or stored under dry conditions. Other investigations have found that the light and temperature stimuli received during development and maturation of seeds on the parent plant can affect their subsequent germination responses. For example seeds of Anagallis arvensis have a very low dormancy when produced in a temperature regime of 30/25°c (day /night), fairly high dormancy at 25/20°c and extreme dormancy at 20/15°c (Grantlipp and Ballard 1963). The role of photoperiod in the initiation of dormancy in several species is well documented (Karssen 1970, Gutterman 1973) and effects on dormancy of the spectral composition of light in which seeds mature have been found in Arabidopsis thaliana (McCullough and Shropshire 1970) and Cucumis spp. (Gutterman and Porath 1975).

These effects are likely to be mediated via the phytochrome system which is able to detect the red:far-red ratio of light reaching the maturing seed. The quality of light reaching the embryo of the maturing seed is influenced by the colour of the surrounding tissues. We would therefore expect the chlorophyll content to be especially important since this pigment, by absorbing red but not far-red light, will determine the red:far-red content of the radiation reaching the enclosed embryo and hence control the proportion of phytochrome established in the active, far-red absorbing form. Seed dormancy in several species studied by Cresswell and Grime (1981) is indeed correlated with the chlorophyll concentration of the maternal tissues around the embryo. Those species in which the chlorophyll level in the investing tissues drops early in seed development tend to produce non-dormant seeds. In contrast, the seeds produced by species whose

extra-embryonic tissues remain green until after the seeds have started to dry are dark-dormant i.e. they need light to induce germination. This is presumably because when shed from the plant they contain an inadequate Pfr:Ptotal ratio.

In order to assess the importance of previous conditions on the dormancy of buried seeds further work must be carried out on the retention of stimuli in the natural environment. Results described in this chapter show that the different effects of pretreatments were rapidly lost when seeds were imbibed in darkness. However, seeds of a winter annual, Draba verna, given a light stimulus before burial in spring became dormant during the high summer temperatures but were able to germinate as soon as temperatures became favourable in autumn. This was in contrast to seeds buried immediately after dispersal which required light for germination and suggests that the initial light stimulus could be retained for several months in the normal wetting and drying cycles found in the surface layers of soil. (Baskin and Baskin 1972).

The mechanism behind the above observations probably involves the dark reversion of Pfr to its inactive form which is known to take place in imbibed seeds in darkness and is more rapid at high temperatures (Taylorson and Hendricks 1969). The retention of a light stimulus by Draba verna seeds could be accounted for if they were rarely imbibed sufficiently for dark reversion of Pfr to take place. We need to know more about how phytochrome dark conversions are affected by various hydration levels before we can draw conclusions.

The results described here for Rumex obtusifolius (Tables 7.4 and 7.5) suggest that imbibed buried seeds become progressively less responsive to a brief light stimulus with increasing time of burial but they remain fairly responsive to large temperature fluctuations.

These findings on the effect of light on dark imbibed seeds are very different from the overall conclusions of Taylorson (1972), who buried weed seeds in the field and then studied their response to light and temperature when exhumed in darkness at three month intervals. He states that the experiments repeatedly demonstrate that burial of initially dormant weed seeds leads to increased germinability and that some seeds become highly sensitive to light. However, this general conclusion ignores the fact that at least one species (Barbarea vulgaris) becomes less responsive to brief exposure to red light during the initial three months of burial and is only particularly germinable when exposed to light at certain times of the year. It is possible that the Rumex obtusifolius seeds studied here would again become more responsive to light if buried in the field where they would be exposed to the normal seasonal changes in absolute temperatures.

Studies in which cyclic changes in dormancy status of buried seeds have been demonstrated (e.g. Courtney 1968) have shown temperature to be the dominant external influence. The lack of response to light after twenty days of dark imbibition could be accounted for by the phenomenon known as skotodormancy. Skotodormancy is characterised by the loss of sensitivity to gibberellin and then to light (Bewley 1980) and is a form of secondary dormancy. This is discussed in more detail at the end of this chapter.

Ecological implications of interactions between stimuli

The results described here show that the response of a buried seed to a particular stimulus such as light or alternating temperatures may well be modified by its past history. Seeds that had previously been exposed to fluctuating temperatures and then dried

were very germinable when given a short light stimulus shortly after re-imbibition compared with seeds having received other pretreatments at constant temperatures (Fig. 7.10). Seeds having received increasing periods of far-red light were subsequently less germinable in a dark fluctuating temperature regime (Fig. 7.5). Increasing exposure to far-red light also increased the time taken for a given proportion of the seeds to germinate (Fig. 7.6). This effect on the rate of germination could have important ecological implications. Newman (1963) has pointed out that for certain species (Aira praecox and Teesdalia nudicaulis) the rate of germination in the summer is generally so slow that the period of continuous moisture required for a high percentage germination rarely occurs in the field. Thus seeds near the soil surface which experience more rapid wetting and drying cycles would be less likely to germinate if they had previously been exposed to a long period of far-red light.

Possible mechanisms underlying the germination responses of buried seeds

The results reported in this chapter, which indicate interactions between alternating temperatures and a light treatment, suggest that a common underlying mechanism may be affected by the two different stimuli. It has been thought for some time that phytochrome acts by altering membrane properties in some way which then leads to changes in transmembrane transport (Smith 1976). The dormancy breaking action of fluctuating temperatures has also been explained by their effect on membrane structure (Hendricks and Taylorson 1979).

A model proposed by Haupt and Weisenseel (in Smith 1976) can be used as a basis to explain many of the germination responses of seeds described in this thesis. They suggest that phytochrome molecules are

only peripherally associated with membranes in the Pr form but extend across them and create aqueous pores when in the Pfr form. This would result in increased transport of hydrophillic substances across the membranes. Increased levels of Pfr may enable particular substances needed for germination to be transported to their sites of action within the seed. Migration of the phytochrome (Pfr) molecules into and out of the membrane matrix would depend on the physical state of the membrane. Thus active phytochrome (Pfr) may be present in a seed but not result in germination until temperature changes enable it to be incorporated into the membrane structure and form aqueous pores.

Seed germination in response to chilling

Work by Van der Woude and Toole (1980) on Lactuca sativa seeds conforms to the above model. They found an enhanced sensitivity to low levels of Pfr caused by prechilling treatments. These treatments were paralleled by decreases in membrane lipid viscosities, which suggests that changes in temperature may have enabled Pfr molecules to be incorporated into the membrane matrix. The rapid flush of seedlings from buried seeds after a short chilling period was described in Chapter 5 (see Figs. 5.11-5.16). These observations may be explained if the period of low temperatures caused an increased sensitivity to the low levels of Pfr already present in the seeds. Such increased sensitivity may then have enabled the metabolic events which end dormancy to occur at higher post-chilling temperatures. The marked synergism between the effects of chilling and light on germination of several weed species reported by Vincent and Roberts (1979) also supports the involvement of membrane structure in the dormancy breaking action of the light promoted form of phytochrome (Pfr).

Skotodormancy

Seeds imbibed in darkness at 18°C for more than two days were found to be virtually irresponsive to five minutes white light (Table 7.5). This lack of response to light characterises a form of secondary dormancy known as skotodormancy, previously thought to be coat-imposed (LeDeunff 1973). Recent evidence from work on Lactuca sativa (Georghiou and Thanos 1983) confirms that skotodormancy cannot be broken by brief exposure to light (ten minutes red light) but shows that it can be broken at 25°C by both continuous and intermittent red irradiation. The involvement of phytochrome in breaking skotodormancy is clearly shown by the red/far-red reversibility obtained in both brief and intermittent irradiation regimes. Although evidence points to the involvement of phytochrome in skotodormancy an underlying mechanism for this phenomenon has not yet been proposed.

SUGGESTIONS FOR FURTHER WORK

Effects of seed moisture content

The results discussed in this chapter point to the importance of seed moisture content in affecting germination responses. Moisture conditions can affect the degree to which an initial stimulus is perceived by the seed, the length of time the effects of that stimulus are retained and the extent to which seeds are able to respond to subsequent stimuli. It has already been noted that several different reactions involving phytochrome may be occuring simultaneously in imbibing seeds exposed to far-red light (see discussion of Fig. 7.5). It is likely that the rates of these reactions would be affected to different degrees if the seeds were maintained in a partly imbibed state, which is frequently the case in nature. The moisture content

necessary for lettuce seeds to perceive red and far-red light has been investigated by Berrie, Paterson and West (1974) who found that some Pfr generation can take place at water contents as low as 15%. We do not know if dark reversion proceeds at lower hydration levels and when it ceases. Answers to these questions could help us to understand how dark-germinability and light-requirement develop.

Apart from the effects of light, temperature fluctuations alone may promote seed germination. Little seems to be known about the minimum moisture content required for temperature shifts to be perceived by the seed. This area has probably been avoided due to the technical difficulties involved. A change in temperature inevitably affects the relative humidity of the atmosphere surrounding a seed and hence its moisture content. In nature buried seeds would experience diurnal temperature fluctuations in soil with a wide range of moisture contents. These conditions could be simulated in the laboratory and the germination responses of seeds to temperature fluctuations in soil maintained at specific percentage moisture levels could be recorded. Thus, although the exact moisture content of the seeds would not be known, qualitative comparisons could nevertheless be made. A promising area for investigation in this connection involves certain seed populations that are known to respond rapidly to single temperature shifts. Frankland (pers. comm.) has found Rumex obtusifolius seeds that respond to single brief high temperature changes. In these cases the metabolic changes caused by the temperature shift may occur before the effects of an external change in relative humidity are felt by the seed. The effects of temperature changes at particular seed moisture contents could therefore be assessed more accurately.

Seed heterogeneity

Some of the germination responses described in this thesis and elsewhere may be masked by the relatively large heterogeneity of weed species. Frankland (1976) in Smith showed that the seed progeny of 20 individual Sinapis arvensis plants gave, in the dark, between 0% and 52% germination, i.e. dormancy was present in 100% to 48% of the seeds, depending on their mother plant. Some of the seed samples responded poorly to ten minutes red light, whereas others showed almost complete breaking of dormancy. This suggests that more accurate comparisons between the effects of different pretreatments could be made using seeds produced from parent plants having the same genetic make-up and grown under identical temperature and lighting conditions. The results of artificial burial experiments would also be more meaningful if the past history of the seed batches was known.

CHAPTER 8

WIDER ECOLOGICAL IMPLICATIONS

The germination requirements of both harvested and buried seeds, obtained using a variety of different methods, have already been discussed in the relevent chapters. In this chapter an attempt will be made to place the phenomena encountered in these studies in a wider ecological context. The possible mechanisms underlying these phenomena will only be mentioned briefly where necessary, as this subject is discussed in detail in Chapter 7.

In the first section of this discussion the germination responses of naturally buried seeds to temperature fluctuations and the adaptive significance of these responses are briefly reviewed (full details in Chapter 5). Differences in germination responses were found when harvested and buried seeds of the same species were compared (Chapter 3). Possible explanations for these differences are suggested and their relevance to the persistence of seeds in a seed bank is then discussed.

Section two deals with evidence relating to the regulation of the timing of buried seed germination by cyclic seasonal changes in the climate (Chapter 4). Finally section three covers the adaptations of species having persistent seed banks to avoid large seedling losses from unpredictable events (eg. fire or a late frost). The possibility of these species having different regeneration niches (see Chapter 6) is related to the maintenance of species diversity in different types of vegetation.

SECTION 1

General responses of buried seeds to temperature fluctuations

The results of experiments on the thermogradient bars using naturally buried seeds (Chapter 5) indicate that several

generalisations can be made about their germination responses to temperature fluctuations. In the field these responses are likely to provide information for the seed about its immediate environment and thus increase the probability of a seedling emerging in optimum growing conditions.

Response to increasing amplitude

The general trend of increasing germination with increasing amplitude of temperature fluctuations probably has adaptive significance. The perception of amplitude could be used by buried seeds as a depth detecting mechanism as the amplitude of temperature fluctuations rapidly drops with increasing distance from the soil surface (Thompson 1977). Lack of germination at small amplitudes would therefore prevent seeds germinating from depths where they would have insufficient seed reserves to reach the soil surface.

This same response could also act as a gap detecting mechanism to prevent seedlings emerging in excessive shade. The amplitude of temperature fluctuations beneath the soil would obviously be reduced as the amount of surface litter and vegetation increased (evidence in Fig.6.1). The above mechanisms have already been suggested from similar results obtained when testing harvested seeds (Thompson and Grime, 1983).

The marked response to temperature fluctuations per se rather than changes in mean temperature is shown by the data in Fig 5.9 for Rumex obtusifolius and Holcus lanatus. The strong inhibition by certain constant temperatures is overcome by the inclusion of these temperatures in a fluctuating regime irrespective of its mean temperature.

Germination in darkness

Subsamples of the soil containing each of the fourteen species tested on the thermogradient bars were exposed to light or maintained in darkness (see Chapter 2 for method). There was generally a substantial increase in germination when light was given, irrespective of temperature. However light could not substitute completely for temperature fluctuations. This general reduction in germination in darkness would again reduce the probability of a seed germinating from excessive depths below the soil surface or beneath a thick litter layer. Both of these would increase seedling mortality.

Response to very large fluctuations

A further rather unexpected response that was recorded for over half the species tested occurred at amplitudes greater than approximately 19°C. Field studies (Fig 4.9) have shown that these conditions may occur near the surface of bare soil in late spring and summer. Results from the thermogradient bars (Figs 5.2-5.8) showed a reduction in germination at large fluctuations (>19°C), particularly when soil was tested in the dark. Field measurements (Fig 4.9) showed that these large fluctuations generally occur after several days drought. It was therefore suggested that a lack of response to high amplitudes of temperature fluctuation could protect seeds from germinating when there would be insufficient moisture for the seedlings to survive. Further evidence in support of this view is given in Chapter 5. It is not known if harvested seeds of these species show a similar response as the temperature regime used by Thompson and Grime (1983) only extended to approximately 12°C amplitude of temperature fluctuation.

The fact that the above generalisations can be made about the germination responses of 14 species of buried weed seeds is not surprising. All buried seeds would have to respond to light and fluctuating temperatures in a similar way in order to germinate in places free from competition. These general responses would also be strongly selected in order to avoid seed germination at adverse microsites (eg those at great depths or those liable to rapid dessication) which would result in seedling death. This relative uniformity of behaviour in these 14 species suggests that the major niche differentiation in seeds is perhaps between species with and without seed banks, with a good deal of heterogeneity within the group lacking seed banks.

However there are exceptions to the generalisations made above. There is a lack of response to increasing amplitudes of temperature fluctuation in Spergula arvensis (Fig 5.8). There is also an obligate requirement for light over the whole range of temperature fluctuations in Juncus acutiflorus (Fig 5.5) and Hypericum perforatum (Fig 5.5). There are minor differences in the shape of many of the germination response curves (Figs 5.2-5.8) particularly at the extremes of the temperature range. These departures from the generalisations described above may have a role to play in niche separation in a heterogenous habitat, where several species having persistent seed banks occur together. Evidence for this is discussed in Chapter 6 and also in section three of this chapter.

Differences in germination response between harvested and naturally buried seeds

The results from germination tests on the thermogradient bars (Chapter 3) showed that naturally buried seeds of Holcus lanatus,

Rumex obtusifolius and Epilobium tetragonum were relatively less stimulated to germinate by diurnal temperature fluctuations of up to about 10°C than were harvested seeds of the same species. This was particularly marked in the dark tests.

Similar differences were also found when the germination responses of naturally buried seeds of other species (Figs 5.2-5.8) were compared with published results for harvested seeds of the same species (Thompson and Grime 1983). Harvested seeds were frequently more germinable at small amplitudes of temperature fluctuation both in the dark and the light. The requirement for temperature fluctuations was abolished by light in several species of harvested seeds (eg Holcus lanatus and Poa annua), but this was not the case for naturally buried seeds of these species.

Acquisition of a fluctuating temperature requirement

The differences in germination response between harvested and naturally buried seeds could be explained by the suggestion that seeds acquire a fluctuating temperature requirement during burial. It has already been shown that seeds can acquire a light requirement during burial (Wesson and Wareing 1969b, Karssen 1981(a)), but the reasons for this are not completely understood. It could be due to anaerobic conditions and anaerobic metabolites in the soil (Wesson and Wareing 1969b, Holm 1972) or to exposure to far-red irradiation before burial (Kendrick 1976, Gorski et.al. 1978). In lettuce seeds a light requirement has been induced by various inhibitory conditions such as water stress (Hsiao and Vidaver 1973; Berrie, Paterson and West 1974) or supra- optimal temperatures (Vidaver and Hsiao 1975). Any of the above factors could also be involved in the induction of a fluctuating temperature requirement.

Evidence has been obtained for Rumex obtusifolius (Chapter 7) which suggests that various stimuli can induce a requirement for fluctuating temperatures in harvested seeds of this species. The results also show that the requirement for fluctuating temperatures and the requirement for light cannot be clearly separated. Seeds imbibed in darkness for several days under conditions unfavourable for germination became almost irresponsive to a short period (5 mins) of white light. This state of light irresponsiveness, known as skotodormancy, could be broken in approximately 50% of the seeds by exposing them to temperature fluctuations in darkness (Table 7.5). Skotodormancy can also be overcome by exposure to prolonged red light (Georghiou and Thanos 1983).

Prolonged exposure to far-red light can be overcome to a limited extent by temperature fluctuations in darkness (Fig 7.5). However in many cases germination would only be brought about by the presence of light. A strong positive interaction between the effects of light and fluctuating temperatures on breaking dormancy has been noted by several workers (eg. Vincent and Roberts 1977, Roberts and Benjamin 1979).

The underlying mechanism suggested for breaking secondary dormancy by active phytochrome molecules (Chapter 7) helps to explain these observations. Active phytochrome may only be able to act effectively when the molecules are incorporated into a membrane matrix (Smith 1976). The physical structure of the membranes would determine whether or not this incorporation could take place. Temperature shifts (and therefore fluctuating temperatures) are known to affect membrane structure and possibly increase the seed's sensitivity to a particular level of active phytochrome. Van der Woude and Toole (1980) found an enhanced sensitivity to low levels of Pfr caused

by prechilling treatments. This effect could account for the rapid flush of seedlings emerging from buried seeds after a short chilling period (Figs 5.11-5.16).

The differences in germination responses between harvested and naturally buried seeds may be at least partly explained by the effects of prolonged dark imbibition while germination is prevented. These conditions may cause the membrane structure to become irresponsive to Pfr and thus be 'inactivated'. Pfr also tends to revert to its inactive form (Pr) when seeds are imbibed in darkness. When these seeds are subsequently exposed to light at more or less constant temperatures the large quantities of Pfr may be able to interact with the membranes in some seeds sufficiently well to cause germination. In other seeds the membranes may need to be 'activated' by temperature fluctuations before the Pfr can take effect.

Alternative explanation for germination responses of buried seeds

There is another possible explanation for the differences in germination response found between harvested and buried seeds. The least dormant seeds in any population may rapidly germinate within the first year after shedding. The buried seeds tested on the thermogradient bars would then represent the more dormant part of the population. These more deeply dormant seeds would require larger temperature fluctuations and often light as well to allow germination. There is a good deal of circumstantial evidence to support this idea. Some of this evidence is discussed below.

Evidence for relatively rapid seed germination after shedding

Seeds may become buried in a number of different ways, including the activities of earthworms or other invertebrates (McRill and

Sagar 1973), and by cultivation or other types of soil disturbance. It must be borne in mind that seeds only remain in the seed bank if they do not rapidly die or germinate. It used to be thought that newly shed weed seeds at sites which were regularly ploughed would be rapidly buried and contribute little to the seedling population the following season (Harper 1957). However mark and recapture studies on Alopecurus myosuriodes seeds (Naylor 1972) indicated that about two-thirds of the plants comprising an infestation were derived from seed less than one year old. Either the mouldboard plough is an inefficient tool for seed burial or these seeds did not have a light requirement imposed by burial.

The experiments of Roberts and Feast (1972) in which freshly harvested seeds of several weed species were artificially buried at different depths also show that many seeds do not stay buried for very long. Appreciable numbers of seedlings of some species (eg. Poa annua, Stellaria media and Spergula arvensis) emerged during the first autumn after burial. When mixed with 2.5cm of soil, between 35% and 85% of the seeds gave rise to seedlings when the soil was disturbed once (in October).

Persistence of seed banks

Thompson and Grime (1979) screened many species and found a continuum of seed bank persistence. They were able to describe four types of seed banks of common occurrence in temperate regions. The main distinction was drawn between transient seed banks (Types I and II) in which no viable seeds remained for longer than a year, and persistent seed banks (Types III and IV) in which there was a carry-over of some viable seeds from year to year. In each of the ten plant communities which Thompson and Grime examined there were some

species which had persistent seed banks, and there were usually others of which viable seeds were present for only part of the year.

Much of this variation in seed bank persistence may be explained by the suggestion that each buried seed is faced with two conflicting selection pressures. Firstly, to delay germination until such time as the microsite conditions are optimal for seedling growth and survival. Secondly, to germinate before the seed dies due to ageing, predation or attack by pathogens. The first of these requirements implies a need for relatively strict control of the location and timing of germination, the second that germination should take place (eventually) whatever the conditions. The balance struck between these two requirements may be partly determined by the innate longevity of the seed. Evidence for this is discussed below.

Long-lived seeds

Baskin and Baskin (1981) studied Verbascum blattaria seeds, known for their long survival in buried seed experiments (Kivilaan and Bandurski 1973). They found that deeply buried seeds could germinate to 82% when exhumed in darkness and exposed to 20/10°C during the first spring after burial. Seeds that were left and exhumed the second spring after burial only germinated to 5% under the same conditions. These data indicate that if seeds do not germinate the first spring after burial they may not be able to germinate in darkness at early spring temperatures in subsequent years.

Species having seeds that are innately longer lived and less prone to pathogen attack could afford to be more selective in when and where they germinate. Certain species which are known for their longevity have an obligate requirement for light. For example, viable seeds of Hypericum perforatum have been found beneath woodland which

has not been disturbed for 170 years (Darby, pers. comm.) and abundant reserves of Juncus species have been found beneath vegetation that does not include mature plants of this species (Thompson and Grime 1979). My observations at a derelict pasture site (Chapter 6) which had not been disturbed for at least 5 years revealed significant numbers of viable Anagalis arvensis and Coronopus didymus seeds but there were no mature plants of these species in the vegetation. Buried seeds of Coronopus didymus tested in the thermogradient apparatus would only germinate when temperature fluctuations exceeded approximatly 10°C. Such fluctuations are unlikely to occur beneath the grassland vegetation at that site.

Short-lived seeds

There is circumstantial evidence to suggest that short-lived seeds are much less selective in when and where they germinate. It is known that the seeds of species having transient seed banks generally show a lack of dormancy mechanisms and an ability to germinate over a wide range of temperatures in both light and darkness (Thompson and Grime, 1979). Exceptionally short-lived seeds which fall in this category (eg. Taraxacum officinale and Tussilago farfara) have the capacity for immediate germination in the laboratory with no specialized temperature requirements (Grime et.al. 1981). More direct evidence was obtained for seeds of Lactuca serriola, which has a relatively short half-life (approximately 1.5 years). Buried seeds of this species rapidly lost a light-requirement for germination, indicating a reduction in the depth of dormancy as the seeds aged (Marks and Prince 1982). Seeds recovered after burial for 8 weeks were able to germinate at a constant temperature (15°C) in the dark, unlike freshly harvested seeds which required temperature

fluctuations. Marks and Prince suggested that although most of the seedlings were likely to die as a result of emergence in unsuitable microsites some may become established and even a low rate of survival would ensure that non-discriminating germination was selected, since all the seeds were otherwise destined to die.

Summarizing in general terms, it could be said that the longer a seed can remain viable the more exacting its germination requirements are likely to be and therefore the less likely it is to germinate beneath undisturbed soil. It seems that buried seeds are adapted to sense several aspects of their environment. These include their depth beneath the soil surface, the severity of competition at the surface and possibly the availability of water. Evidence that seeds can also judge the best time for germination in view of the prevailing climatic conditions is discussed below.

However it must be remembered that the interests of the seed and of the mother plant do not always coincide. One might expect that the fitness of individual seeds would be maximised by possession of the same optimal germination requirements. In a fairly uniform habitat this would lead to germination of most or all of the seeds in a persistent seed bank after soil disturbance or when temperature fluctuations exceed a certain threshold. This behaviour could lead to the majority of potential adult plants of a particular population being wiped out by a single unexpected event such as a late frost or a herbicide treatment. A more effective strategy to maximise the fitness of the parent plant would involve intermittent germination of at least a small proportion of the seeds in the seed bank. Much of the evidence presented in this thesis shows that the germination responses of buried seeds to various stimuli are very heterogenous,

and some of this heterogeneity is probably imposed by the parent plant. This would bring about intermittent germination in the field. Some of the environmental factors responsible for increasing this existing heterogeneity are discussed in Section 3.

SECTION 2

Evidence for seasonal emergence from seed banks

It would obviously be advantageous for seedling survival if germination occurred when the climate was favourable for rapid establishment and growth. Thus seedlings could benefit from the temperature and moisture conditions in spring and autumn but avoid the damaging droughts and frosts in summer and winter. There is much evidence that this is in fact the case.

Several workers have described increases and decreases in dormancy in buried seeds which are often part of cyclic changes that follow a seasonal pattern (eg. Stoller and Wax 1974, Roberts and Lockett 1978, Karssen 1981). In seeds of summer annuals, e.g. Polygonum persicaria, induction of secondary dormancy occurs in late spring and early summer during the rise of soil temperature. Dormancy is broken during low temperatures in winter (Karssen 1981). Seeds of winter annuals respond in the opposite way to high and low temperatures. Thus low temperatures inhibit after-ripening and induce secondary dormancy in Veronica hederifolia (Baskin and Baskin 1978).

Secondary dormany prevents the germination of buried seeds during the season preceding the unfavourable conditions for growth and development and, therefore, has great survival value. During the unfavourable season dormancy is released, resulting in the ability to germinate under a wide range of conditions (Vegis 1964). The

evidence from tests on the thermogradient bars (Chapter 4) supports these ideas. The great changes in the total number of seedlings of a particular species emerging on different sampling dates (eg. Polygonum aviculare, Fig 4.6) probably reflect seasonal changes in dormancy. As buried seeds pass through seasonal cycles of imposition and alleviation of secondary dormancy different proportions of the population will be germinable.

Possible mechanism underlying seasonal emergence

The seasonal cycles of dormany described above suggest that seeds respond to changes in absolute temperatures. Vegis (1964) suggests that these responses result in a widening and narrowing of the range of environmental conditions which are suitable for germination. In most controlled burial experiments germination is only tested at one set of alternate or constant temperatures so gradual changes in the range of temperatures needed for germination cannot be seen. However Baskin and Baskin (1980) state that exhumed Ambrosia artemisiifolia seeds gradually lost their ability to germinate in light over a range of pairs of alternating temperatures from April onwards. The results from the thermogradient bars suggest that at least in some species dormancy is an 'all or nothing' phenomenon; although the proportion of dormant seeds may show great seasonal variation, the response of the non-dormant fraction of the population to temperature fluctuations is always similar.

Thompson (1977) found that a large number of species with seeds banks could be germinated in relatively constant numbers from soil samples collected at anytime of the year, with rare exeptions. He suggests that species with seasonal cycles of dormancy may be relatively uncommon but are reported in the literature because they

are interesting. He also suggests that the interaction of a constant germination requirement with a seaonally variable climate would be enough to ensure correct germination timing. This may be true for some species but the evidence presented here suggests that a proportion of the buried seeds in several species "switched off" at certain times of the year. The experiment in which there was a marked effect of chilling on stimulating buried seed germination (Figs 5.11-5.16) supports this. It may be that certain absolute temperatures inactivate the membrane systems in seeds and specific stimuli such as chilling, high temperatures or sometimes drought (Kivilaan 1975) are needed to reactivate them.

Smaller intermittent flushes of emergence

It has already been stated that a very narrow timing of seedling emergence for all seeds of a particular species could lead to massive seedling mortality from unpredictable events.The results suggest that not all the seeds in a population are "switched on or off" by changes in absolute temperatures in the environment(Figs 4-2-4.8).

Many annual weeds show main seasonal flushes of germination with smaller intermittent flushes between these (Roberts and Feast 1970). The smaller flushes may be explained in part by heterogeneity in the seed microenvironment. At certain microsites dormancy could be broken when positively interacting stimuli such as light, large temperature fluctuations and high nitrate levels occur together (Vincent and Roberts 1977, Roberts and Benjamin 1979). This interaction effect could overcome the normally inhibitory effects of absolute temperatures.

SECTION 3

Heterogeneity in time and place of emergence from seed banks

There are several factors that affect the time and place of buried seed germination in the field. These include both intra- and interspecific heterogeneity in germination responses to different environmental stimuli and heterogeneity in microsite characteristics. These are discussed below, but the influence of the horizontal distribution of seeds in the soil, which was found to be highly irregular (Fig. 6.5), has already been discussed in Chapter 6 and need not be mentioned here.

Intraspecific variation in germination responses

Relatively small variations in the response of seeds in a population to stimuli such as temperature fluctuations would ensure variation in the timing of germination in the field. Some intraspecific heterogeneity has been found in the responses of harvested seeds to temperature fluctuations (Thompson and Grime 1983). However, this heterogeneity was greatly increased in naturally buried seeds of the same species (Chapter 3 of this thesis).

The intra-specific variation between harvested seeds can largely be accounted for by small differences in maturity of the seeds when harvested. This would affect the state of the phytochrome system and hence their germination responses (Cresswell and Grime 1981). The results in Chapter 7 shed some light on why naturally buried seeds should be more variable than harvested seeds. Harvested Rumex obtusifolius seeds were able to 'remember' previous stimuli such as exposure to far-red light or several cycles of a fluctuating temperature regime during long periods of dry storage. This was

measured as significant differences in the number germinating in subsequent test conditions (9/25°C dark) when reimbibed (Table 7.2). The differences were less marked when the seeds had been imbibed in the dark for 21 days at 18°C (this prevented germination) before being transferred to the test conditions (Table 7.3). The effect of these initial stimuli appears to be cumulative, for example the longer seeds are exposed to far-red light the less responsive they then become to subsequent stimulatory conditions such as fluctuating temperatures (Fig. 7.5).

Freshly harvested seeds would only have opportunity to respond to environmental stimuli while on the mother-plant (Grantlipp and Ballard 1963) whereas buried seeds may be affected by the density of the plant canopy under which they lie before burial (Fenner 1980, Frankland and Poo 1980) or by periods of fluctuating temperatures after burial. The moisture content of each individual seed will affect its response to light quality (Fenner 1980) and temperature fluctuations (evidence in Chapter 7 of this thesis). There is therefore much scope for buried seeds to acquire germination requirements outside the range found within a harvested seed population of that species.

These observations suggest that the diversity of possible past histories of individual seeds may be important in spreading the timing of buried seed germination in the field.

Heterogeneity in microsite characteristics

In addition to the heterogeneity of the seeds themselves, variation in the time and place of seed germination is also partly controlled by variation in the physical characteristics of microsites within the habitat. Small scale variations in amplitude of

temperature fluctuations, soil moisture, light intensity and leaf shade would be common even at sites which appeared to be very uniform.

Harper, Williams and Sagar (1965) clearly showed that seeds may respond to quite small microhabitat variation. Three species of plantains (Plantago spp.), when sown in seed beds, responded differently to the variations in the environment produced by slight depressions, by squares of glass placed on the soil surface, and by small vertical walls of glass or wood. The differences between microhabitats artificially imposed by Harper et.al. would be difficult to measure and quantify but must obviously have been perceived by seeds of the three Plantago species.

In a natural habitat the physical environment may change significantly over short distances. Thus Fig. 6.1 shows differences in temperature at the soil surface on the same dates at two microsites separated by only a few centimetres. Grime and his colleagues (1982) found striking differences in patterns of germination between the north- and south-facing slopes in the same valley. Such effects of topography may also be felt on a much smaller scale.

Interspecific heterogeneity in germination responses

All of the fourteen species studied here may be found in similar sorts of habitats which suffer from unpredictable and often severe disturbance. They therefore show 'weedy' adaptations which enable the plant population to survive such disturbances as buried seeds and to germinate following them. These adaptations include a persistent seed bank and the generally similar germination responses to light and fluctuating temperatures which have been discussed above. This relative uniformity in germination responses between species is to be expected and reasons for the minor differences in response to

fluctuating temperatures (Figs. 5.2-5.8) are often not easy to identify, although in some cases the adaptive significance is fairly clear.

Obviously it is advantageous for particularly small seeded species (e.g. Juncus acutiflorus) not to respond to temperature fluctuations in darkness. The various shapes of the response curves (Figs. 5.2-5.8) may also be useful in allowing several species with persistent seed banks to exist in more natural vegetation suffering much less disturbance. For example, the south facing site at Litton Mills (Thompson and Grime 1979) supports many different species with both transient and persistent seed banks. Generally the species with transient seed banks (e.g. Festuca ovina and Koeleria cristata)are most common in the vegetation but there are significant numbers of plants with persistent seed banks such as Plantago lanceolata, Holcus lanatus and Origanum vulgare.

At such sites, where there is no overriding selection pressure to surivive major disturbances, there is likely to be much more scope for specialisation on different sorts of relatively rare disturbance. Thus species with transient seed banks may be adapted to exploit seasonally predictable damage by drought. In southern Europe, disturbance of vegetation by drought occurs each summer and is most severe in its effect upon grasses, many of which are shallow-rooted. Under such conditions detached viable seeds of species with transient seed banks are present in the habitat only during the dry season, and reach a minimum in the wet season, when germination results in the appearance of large numbers of seedlings in the areas of bare ground developed during the preceding summer.

Species with persistent seed banks are adapted to exploit unpredictable damage to the vegetation such as that caused by grazing

or trampling by animals. Relatively small differences in germination responses (e.g. with respect to temperature fluctuations) may then enable different species to take advantage of almost undetectable differences between microsites.

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Plate 1

Plate 2

Plate 3

Plate 4

Aspects of Applied Biology 4, 1983
Influence of environmental factors on herbicide performance and crop and weed biology

Germination responses of naturally-buried weed seeds to diurnal temperature fluctuations

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Summary Some results are reported from the use of a thermogradient apparatus which enables soil, containing naturally-buried weed seeds, to be exposed to a range of diurnal temperature fluctuations in both light and darkness. Germination is at least partially inhibited by darkness in all ten of the weed species studied and stimulated by temperature fluctuations in nine. In some species germination is inhibited by very large fluctuations. There is some evidence that a requirement for fluctuating temperatures may be induced by burial.

The implications of the results for the location and timing of weed seedling emergence are discussed. It is suggested that the observed responses to temperature are likely to restrict germination to spring and autumn in most species. It is predicted that emergence of the grassland weed Holcus lanatus will largely be restricted to gaps in the vegetation canopy and this prediction is tested in a field experiment.

INTRODUCTION

The germination requirements of buried weed seeds determine the location and timing of germination of those weeds, and thus the association of particular weeds with certain crops and cropping systems. It is therefore apparent that a knowledge of the germination requirements of naturally-occurring buried seed banks has an important bearing on weed control. Much information is already available on the germination requirements of harvested and stored weed seeds (Grime, Mason, Curtis, Rodman, Band, Mowforth, Neal and Shaw 1981, Thompson and Grime 1983) and in some cases very detailed experiments have been carried out on the interacting effects of several environmental variables on the germination of important weeds (Vincent and Roberts 1977, Roberts and Benjamin 1979). The effects of artificial burial on harvested weed seeds has also received considerable attention (Roberts and Lockett 1975, Roberts and Neilson 1982 a,b). An area which has been relatively neglected, however, is the germination requirements of naturally-occurring populations of buried weed seeds (Wesson and Wareing 1969). There is evidence that changes in the germination requirement of weed seeds may be induced by conditions not only after but also before burial (Fenner 1980).

The aim of this paper is to report some preliminary results obtained from the use of an apparatus designed to subject naturally-buried seeds to various amplitudes of temperature fluctuation in either light or darkness. An attempt is then made to assess the practical significance of the results and to compare the germination behaviour of fresh and buried seeds.

MATERIALS AND METHODS

The apparatus is described in detail elsewhere (Thompson and Whatley, in press) and is in principle a modification of the thermogradient bar described by Grime and Thompson (1976). Two large aluminium plates were each equipped with a water jacket at opposite ends and the water flowing through the jackets was arranged to provide a diurnal temperature fluctuation of 1.5°C (about a mean temperature of 12°C) at one end of each plate and a fluctuation between 6°C and 31°C at the other. The lower temperature was maintained for 14 hours in every 24. Surface soil containing a natural seed burden was collected and dried in darkness, sieved and spread in an even 5mm layer on both bars and subsequently rewetted using a fine mist sprayer. Soil used in the experiments reported here was collected from arable fields and derelict pastures near Plymouth Polytechnic Experimental Station at Rumleigh (Nat.Grid Ref. SX 443 679). Soil on one plate was maintained in darkness by a combination of dim lighting (4.5 Wm^{-2}) and a covering of sterile sand. Light intensity at the soil surface on the other plate was 95 Wm^{-2}. The soil was prevented from drying out by a slow flow of water-saturated air and seedlings were harvested and identified after 14 days. A full discussion of how far the behaviour of buried seeds on the plate can be assumed to reflect their behaviour *in situ* in the field is presented in Thompson and Whatley (in press).

RESULTS

Statistically significant numbers of seedlings were recorded for fourteen species, but only ten of these were important weeds of arable land or pasture. In order to express the distribution of seedling emergence graphically each plate was divided into six longitudinal compartments and these were treated as replicates. Each compartment was divided into five sections, each with the same increment in the number of degrees of temperature fluctuation experienced. Each section could then be assigned a mean amplitude of temperature fluctuation. Since the sections were of unequal area, the numbers of seedlings were expressed on a per unit area basis (500 cm^2). The results for all ten important weeds encountered are shown in Figs. 1 and 2. In all the species germination was enhanced by light, although the difference between light and dark was not always significant. In all species except *Spergula arvensis* (corn spurrey) there was also a significant increase in germination with increasing diurnal temperature fluctuation. This increase in germination was always found at the lower fluctuations (i.e. over the range 5-10°C) but responses varied at higher fluctuations. In some cases, e.g. *Plantago major* (great plantain) in both light and darkness and *Polygonum aviculare* (knotgrass) in darkness only, increased germination was elicited by fluctuations up to 25°C, the largest employed. In other cases, e.g. *Coronopus didymus* (lesser swine-cress), there was little or no significant change in germination above a fluctuation of 10°C. A third type of response was shown by *Stellaria media* (chickweed), in which large fluctuations dramatically reduced germination to a level similar to that at the lowest fluctuation.

DISCUSSION

It is apparent that stimulation of germination by light is a ubiquitous feature of the buried seeds of all the weed species studied here. This connection between accumulation of a persistent reservoir of buried seeds and at least partial inhibition of germination by darkness has been noted previously (Thompson & Grime 1979). It is this requirement for light which results in the flushes of weed seedlings observed after soil disturbance. It is still not clear whether this dark inhibition arises during burial or is an innate

Fig.1 The response of naturally-buried seeds to diurnal temperature fluctuations in light (o) and darkness(o). (a) Poa annua, (b) Spergula arvensis, (c) Stellaria media, (d) Cardamine hirsuta, (e) Coronopus didymus. Vertical bars represent the least significant differences (P = 0.05) between treatment means in light (upper bar) and darkness (lower bar).

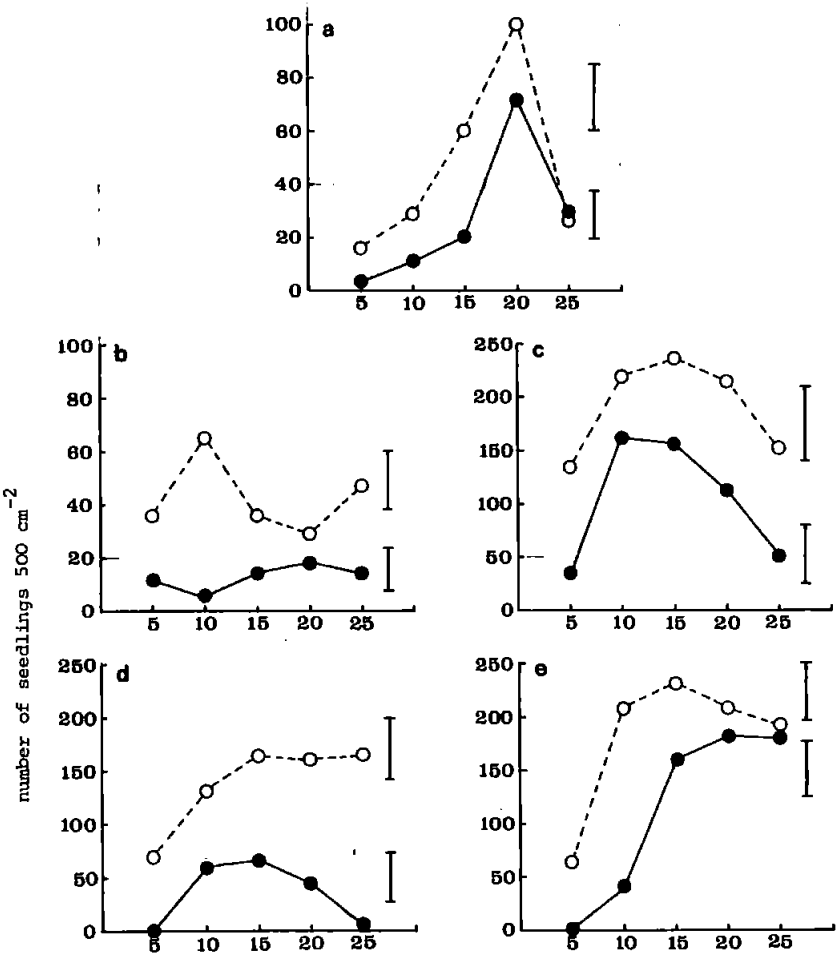
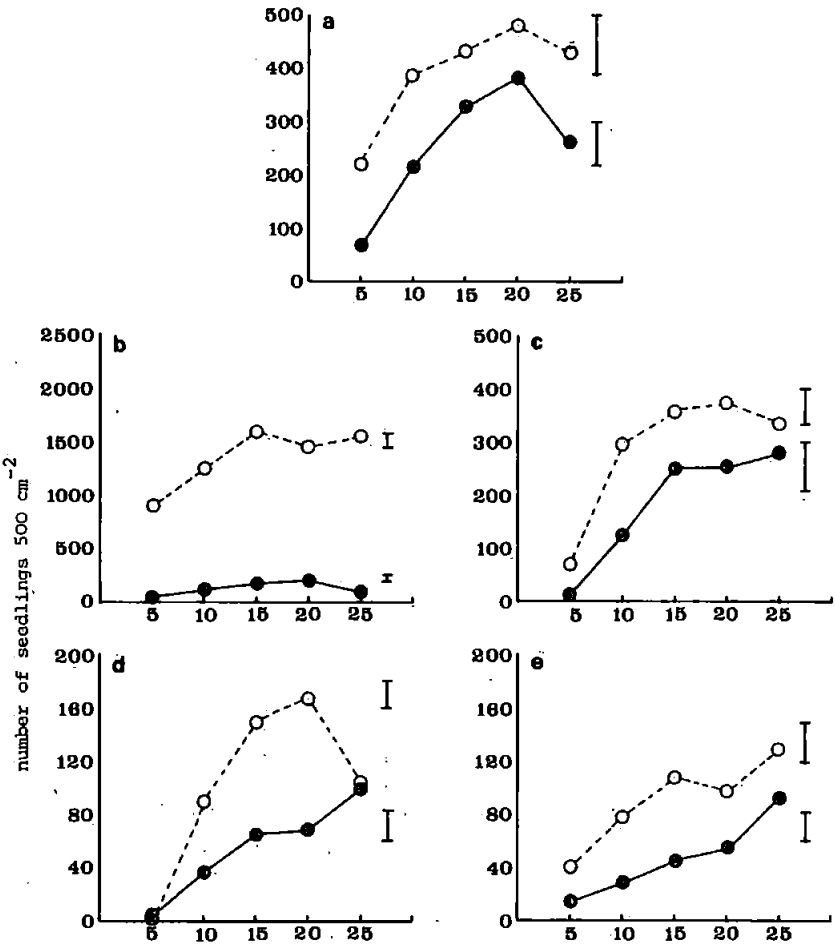


Fig.2 The response of naturally buried seeds to diurnal temperature fluctuations in light (o) and darkness (o). (a) Holcus lanatus, (b) Epilobium tetragonum, (c) Rumex obtusifolius, (d) Polygonum aviculare, (e) Plantago major. Vertical bars represent the least significant differences (P = 0.05) between treatment means in light (upper bar) and darkness (lower bar).



feature of the freshly shed seed. Contradictory reports of dark inhibition of germination in weed seeds are commonplace and derive variously from ecotypic variation, differences in age and storage conditions of seed and even the degree of ripeness of seed when harvested (Cresswell and Grime 1981). An interesting point is that by far the greatest dark inhibition recorded here was in Epilobium tetragonum (square-stemmed willowherb) which like all willowherbs has an extremely small seed. That such a large number of Epilobium seeds germinated in the light (equivalent to a seed bank of 600,000m^{-2} in the top 10cm of soil) is, furthermore, good evidence that light penetrated all or nearly all of the 5mm depth of soil on the bar exposed to light. It therefore seems that the stimulation of germination by fluctuating temperatures in the light, found in most of the species studied, is a real phenomenon and not an artefact due to limited light penetration.

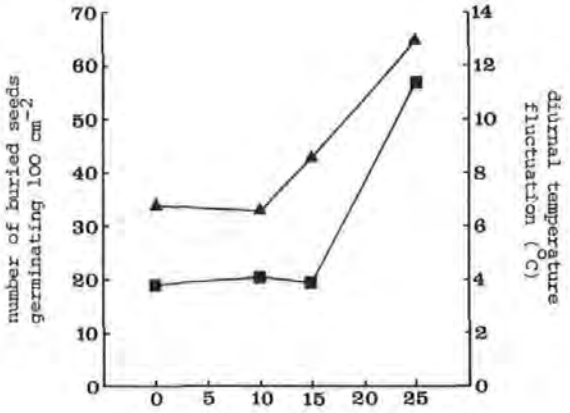
Unlike light, the ecological significance of germination stimulation by fluctuating temperatures is potentially rather complex (Thompson & Grime 1983). The weather, the season, depth of burial and degree of plant and litter cover will all influence the amplitude of fluctuation experienced by a buried seed. In practice arable weeds will normally germinate in relatively bare earth and therefore the last of these complications can be ignored. A requirement for relatively large fluctuations (>10°C), found in the weeds studied here, will prevent germination until the soil begins to dry out and warm up in spring. It is therefore of interest to note that in the vast majority of important British weeds, rather little germination takes place before March (Fryer and Evans 1968, Roberts and Feast 1973) and the bulk of germination occurs in March, April and May. Naturally there will be year-to-year variation in the precise pattern and germination will be delayed in cold, wet springs.

In several of the species studied here germination is inhibited by very large fluctuations, which are likely to occur when insolation is high and soil moisture is low. This would tend to prevent germination in June and July, and in fact almost no weeds of any importance show significant germination during this period (Fryer and Evans 1968, Roberts and Feast 1973). The selective value of such germination behaviour is obvious - seedlings appearing at a time of drying soil and high temperature are likely to fall victim to drought. In the autumn, soil temperature fluctuations are again lower and one would expect a second period of germination. It is therefore not surprising that one of the most typical patterns of germination in British arable weeds, shown for example by Poa annua (annual meadow grass) and Stellaria media, is two peaks in spring and autumn with few seedlings appearing in summer.

Germination in weeds of pasture is complicated by the insulating effect of various depths of litter and vegetation. These greatly reduce soil temperature fluctuations and therefore tend to restrict germination to gaps in the vegetation. In Holcus lanatus (Yorkshire fog) for instance, most seeds at the soil surface require fluctuations of 10°C or above, while many buried seeds appear to require fluctuations of 15°C or more. One would therefore predict that buried Holcus lanatus seeds would only germinate in vegetation gaps experiencing relatively large temperature fluctuations. In order to test this prediction circular gaps of varying diameter were cut in the vegetation of a perennial ryegrass ley during early April (Thompson 1977). Both emergence of Holcus lanatus seedlings from buried seeds and soil temperature fluctuations at a depth of 1cm were recorded in the gaps and in undisturbed vegetation. It is clear from Fig.3 that in small gaps and beneath undisturbed canopy fluctuations were of the order of 7-9°C and germination was relatively low. In large gaps, experiencing mean fluctuations of <13°C, and peak fluctuations well in excess of this, germination was increased almost threefold. It is likely that a similar 'gap-detecting' mechanism exists in other weeds of pasture, e.g. Poa trivialis (rough meadow grass), Poa pratensis (smooth meadow grass) and Deschampsia caespitosa (tufted hair grass), in all of which seeds are known to respond to fluctuating temperatures in darkness (Thompson and Grime 1983).

The practical lessons for grassland management are important. Any

Fig. 3 Diurnal soil temperature of fluctuations (▲) and germination of buried Holcus lanatus seeds (■) in artificial canopy gaps in perennial rye-grass pasture. Temperature fluctuations are the mean amplitudes of diurnal fluctuations measured in the centre of circular canopy gaps at a soil depth of 1 cm from 11-23 May. Germination counts are sums of several counts carried out from 6 April to 30 May.



management of rye grass pasture which results in the creation of gaps in the vegetation (e.g. damage by machinery or animals, herbicide treatment) is likely to lead to the invasion of weed grasses from the seed bank. Such invasion should be reduced by keeping damage to the sward to a minimum and restricting such damage to periods when germination of weed seeds is prevented by drought or high temperatures.

It has already been noted (Thompson and Grime 1979, 1983) that stimulation of germination by fluctuating temperatures in darkness is a frequent feature of harvested, dry-stored weed seeds. One might therefore have predicted some of the results discussed above, at least qualitatively, from work on dry-stored seed. What is more surprising is the almost universal stimulation of germination by fluctuating temperatures in the light. Specifically, experiments on several different lots of dry-stored Poa annua and Holcus lanatus seeds (Thompson 1977, Thompson and Grime 1983) have failed to reveal any evidence of such a response and germination in these species in the light occurs over a wide range of constant temperatures. It therefore seems that not only may seeds acquire a light requirement before or during burial (Wesson and Wareing 1969, Fenner 1980), they may also acquire a requirement for fluctuating temperatures in the light as a consequence of burial.

ACKNOWLEDGMENTS

We would like to thank Dr. E.A. Vallis and Dr. G.D. Wigham for their advice while designing the apparatus and Mr. L.F. Cook and Mr. A. Hopkins for technical assistance. This work was supported by a Devon County Council research assistantship to J.C. Whatley.

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A THERMOGRADIENT BAR APPARATUS FOR THE STUDY OF THE GERMINATION REQUIREMENTS OF BURIED SEEDS *IN SITU*

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(Accepted 21 October 1983)

SUMMARY

An apparatus is described which enables a layer of soil, containing naturally-buried seeds, to be subjected to controlled temperature fluctuations over the range 5 to 30 °C, in the presence or absence of light. Results obtained from the use of this apparatus are presented for 14 species, all common constituents of the buried seed bank and nearly all weeds of arable land or pasture. At least partial inhibition of germination by darkness was found in all 14 species and stimulation of germination by temperature fluctuations in all but one. In some cases germination was inhibited by very large fluctuations.

There was some evidence that a requirement for temperature fluctuations may be induced by burial. The ecological significance of the results and the limitations of the thermogradient apparatus are discussed and some further applications of the apparatus are suggested.

Key words: Thermogradient bar, buried seeds, germination, weeds, fluctuating temperatures.

INTRODUCTION

A thermogradient bar consists of a bar of heat-conducting material which is heated at one end and cooled at the other, thus producing a gradient of temperature along the length of the material. The whole apparatus is thoroughly insulated against heat loss, which could otherwise cause the gradient of temperature to be uneven or promote a gradient of temperature across the width of the bar. These bars have been used in the determination of temperature optima and limits for insects (Coulianos, 1955), algae (Halldal & French, 1958), seed germination (Larsen, 1965; Wagner, 1967) and root growth (Barbour & Racine, 1967). More recently they have been used to provide a range of diurnal temperature fluctuations (Grime & Thompson, 1976) and much standardized information is now available concerning the germination responses of many species to different amplitudes of temperature fluctuation (Thompson & Grime, 1983).

The direct application of this information to seed germination in the field is complicated by the observation that fresh and buried seeds may differ in their germination requirements (Wesson & Wareing, 1969; Taylorson, 1972; Karssen, 1981a).

In addition, it is known that a number of factors interact to affect germination of seeds in the soil, including temperature, light, oxygen concentration, allelopathic inhibitors and moisture (Karssen, 1981b). In particular, it is very difficult in field experiments to separate the effects of temperature and moisture. Many workers agree that, after the spring flush of germination, rainfall has an overriding influence on the timing of seedling emergence (Stoller & Wax, 1973; Egley & Williams, 1979;

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Roberts & Potter, 1980). Others have found little effect of soil moisture (Yamamoto & Ohba, 1977) and point to diurnal temperature fluctuations as the main controlling factor (Watanabe & Hirokawa, 1975). The picture is further complicated by annual cycles of increasing and decreasing dormancy (Taylorson, 1970, 1972; Stoller & Wax, 1974; Roberts & Lockett, 1978), a process which is unique to buried seeds and does not occur during dry storage (Karssen, 1981b). Clearly there is a need for controlled experiments, in which the effects of individual factors on buried seed germination can be studied under conditions approximating to those in the field. This paper describes some experiments using an apparatus which enables a layer of soil, containing naturally-buried seeds, to be subjected to controlled temperature fluctuations and moisture in the presence or absence of light.

THE THERMOGRADIENT APPARATUS

Principle and construction

The aim was to subject a layer of soil to a controlled range of diurnal temperature fluctuations, these fluctuations occurring about an intermediate constant temperature. The principle of the apparatus was identical to that described by Grime & Thompson (1976). The main features of the equipment are illustrated in Figure 1. An aluminium sheet, 95 × 95 cm and 0·5 cm thick, was supported on glass-fibre insulation on top of a 7 cm thick sheet of expanded polystyrene. At each end the aluminium sheet projected for 2·5 cm through a water-tight seal into a water-jacket. At the end designed to maintain a constant temperature, the water circulated through the water-jacket was pumped from a cryothermostat with a working temperature range of −10 to +100 °C and a stability of ±0·01 °C. The flow rate was 18 l min^{-1}. At the opposite end of the sheet the water-jacket was connected to a second cryothermostat. This was modified so that the temperature of the water was controlled by an electronic thermostat with two variable temperature settings. The timing of alternations between the two temperature settings was by means of a programmable electronic timing device. A wood framework divided the surface of the aluminium sheet into six identical strips which served as replicates. This provided a chamber around each strip of soil (0·5 cm thick), with internal dimensions of 90 cm long, 15 cm wide and 7 cm tall. A bank of four 30 W 'warm-white' fluorescent tubes was situated 40 cm above a perspex tank which was filled with water and acted as both a heat filter and a lid for the growth chambers. Light intensity at the bar surface was 95 W m^{-2}. Daylength was regulated by the same timer which controlled the cryothermostat. Air was pumped into the assembly of six chambers at one corner and left at the opposite corner, passing from one chamber to another through holes spaced along the top of the wooden partitions. To prevent the soil drying out, the air was first saturated with water vapour by pumping it through a Dreschsel bottle containing water which stood in a water bath maintained at 12 °C. In order to minimize its effect on the temperature gradient the flow rate of air was slow. Measurements of temperature along the bar (Figs 2 and 3) showed that even at the 'constant' end, soil normally experienced a small fluctuation in temperature (1·5 °C).

Method of operation

Adjustments of the thermostats and the time-switch provide for considerable flexibility with regard to the choice of treatments. Recent experiments have used a regime in which the 'constant' temperature maintained at one end of the bar

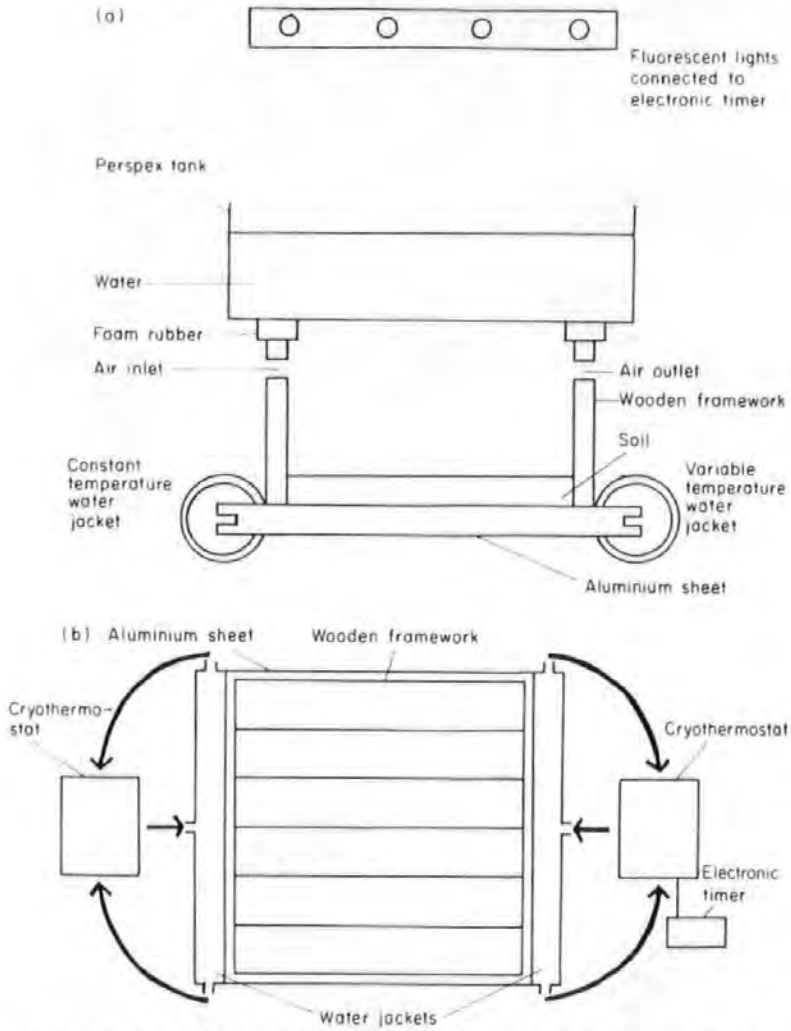

Fig. 1. (a) Side view and (b) top view of thermogradient bar. Arrows represent direction of water flow.

surface was 12 °C and fluctuations over the range 1·5 to 25 °C were induced by depressing the water temperature at the opposite end from 32 to 4·8 °C for 14 h, including a dark period of 10 h (Figs 2 and 3). In practice the apparatus was constructed so that two identical thermogradient bars could be operated in parallel. The second bar was identical to that described above, but for the absence of the fluorescent tubes and heat filter, and was used as described below for the simultaneous testing of soil samples in total darkness. The air temperatures in the light and dark chambers never differed by more than 2 °C and this was insufficient to alter the soil temperatures, which were therefore identical on the two bars. The second bar was positioned 25 cm vertically below the first and was thus out of direct illumination. The diffuse light reaching the soil surface on this bar was therefore only 4·5 W m^{-2}. Soil samples, to be used on both thermobars, were collected in the dark by means of a light-proof tent and then dried in the dark. It has been shown that seeds containing less than 6 % moisture are insensitive to light, due

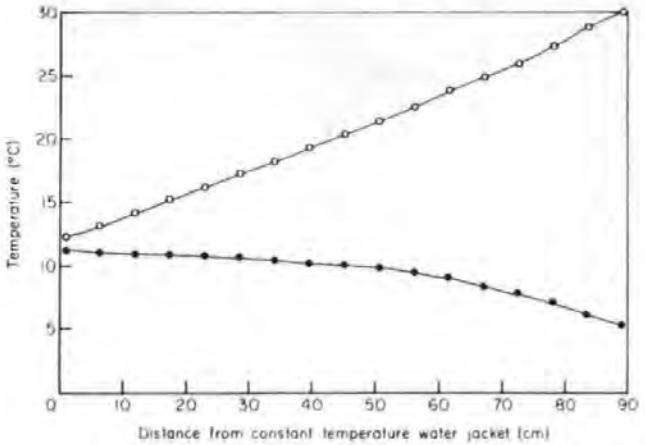

Fig. 2. Temperature gradients at the bar surface during operation of the regime used in current experiments. Gradients maintained for 10 h (○) and 14 h (●) in every 24. A 10 h dark period was included in the latter period.

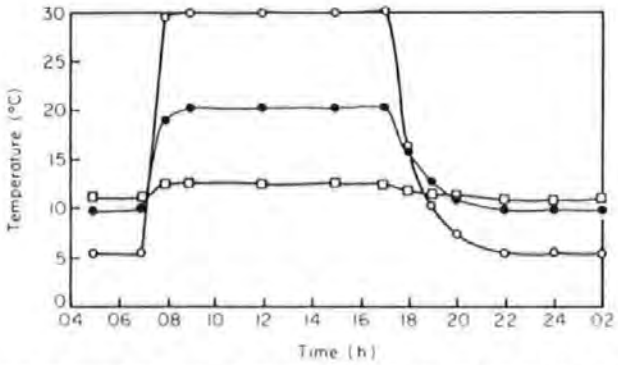

Fig. 3. Diurnal temperature changes recorded on the bar surface at 1 cm (□), 45 cm (●) and 89 cm (○) from the 'constant' end of the bar.

to inactivation of the phytochrome system (Berrie, Paterson & West, 1974; Kendrick, 1976). Manipulations of the dry soil could therefore be carried out in the light as the seeds were 'fixed' with respect to the action of light. The soil was passed through a 3 mm sieve to remove gravel and large roots. This soil was now suitable for germination tests to be carried out in the light but experience showed that most soils did not contain sufficient seed to provide a statistically significant number of germinations in the dark. Accordingly, seeds in the soil to be tested in the dark were concentrated by passage through a second sieve to remove the $< 300\ \mu$m soil fraction. Soil was spread in an even 5 mm thick layer in each growth chamber and that to be tested in the dark was covered with a 2 to 3 mm layer of sterile sand. Controlled experiments have shown that the low intensity of light reaching the chambers was excluded sufficiently by the sand to prevent triggering of seed germination. Other possible effects of the sand are mentioned in the discussion. The soil was then wetted evenly with a fine mist sprayer to a moisture content of 30%. This was checked with a specially designed probe attached to a wheatstone bridge. The soil could be maintained at this moisture content without rewetting by a slow stream of saturated air flowing over the surface. The optimum

time for running the thermogradient bars before plotting the total number of seedlings which had emerged was found to be 14 d for the species tested. There was only a 3 to 4 % increase in total numbers after a further 7 d.

Methods

Soil samples to be tested on the bars were collected from a number of arable fields and derelict pastures near Plymouth Polytechnic Experimental Station (National Grid Reference SX443 679). All soils collected were known to contain persistent buried seed banks of species in which germination of harvested seeds is stimulated by fluctuating temperatures either in the light or dark or both (Thompson, 1977; Thompson & Grime, 1983). Soils were collected and prepared as described above and tested using the temperature regime shown in Figures 2 and 3. This regime approximates to that experienced by surface soil under field conditions in late spring.

For two of the species found in the soil samples (*Rumex obtusifolius** and *Holcus lanatus*) experiments were also conducted using harvested seeds, previously stored dry at 5 °C for several months. Seeds of these two species were placed on a layer of sterile compost on the thermogradient apparatus and germination recorded. Seeds to be tested in the dark were covered with a layer of sand as described for the naturally-buried seeds.

Results

In order to express the distribution of seedling emergence graphically the six chambers on each thermogradient bar were divided into five sections, each with the same increment in the temperature fluctuation experienced. Each section could then be assigned a mean amplitude of temperature fluctuation. Since the sections were of unequal area, the numbers of seedlings were expressed on a per unit area basis. The numbers of seedlings on the light bar were adjusted where necessary to take account of the increased concentration of seeds in the soil on the dark bar. Sufficient seedlings were found to enable statistically testable conclusions to be drawn in a total of 14 species. Results for all these species are shown in Figures 4 to 7. Figure 7 also contains the results for harvested seeds of *R. obtusifolius* and *Holcus lanatus*.

Responses to temperature fluctuations in the light

Stimulation of germination by temperature fluctuations was found in at least 12 of the 14 species examined. In the majority of species the effect of large fluctuations was merely to increase the number of germinations found at the lowest fluctuation (4·8 °C) but in one, *Polygonum aviculare* [Fig. 4(c)], there was an obligate requirement for fluctuations in excess of this value. Several species required large fluctuations [e.g. >9 °C in *Agrostis stolonifera*, Fig. 5(a), and *Poa annua*, Fig. 4(a)] to achieve 50 % of the maximum germination recorded. Conversely in other species [e.g. *Epilobium tetragonum*, Fig. 5(b), and *Stellaria media*, Fig. 4(b)] 50 % of maximum germination was attained at the lowest fluctuation.

Over half of the species examined showed either no further increase or a marked decrease in germination at fluctuations beyond 19 °C. The heterogeneity of

* Nomenclature follows Clapham, Tutin & Warburg (1962).

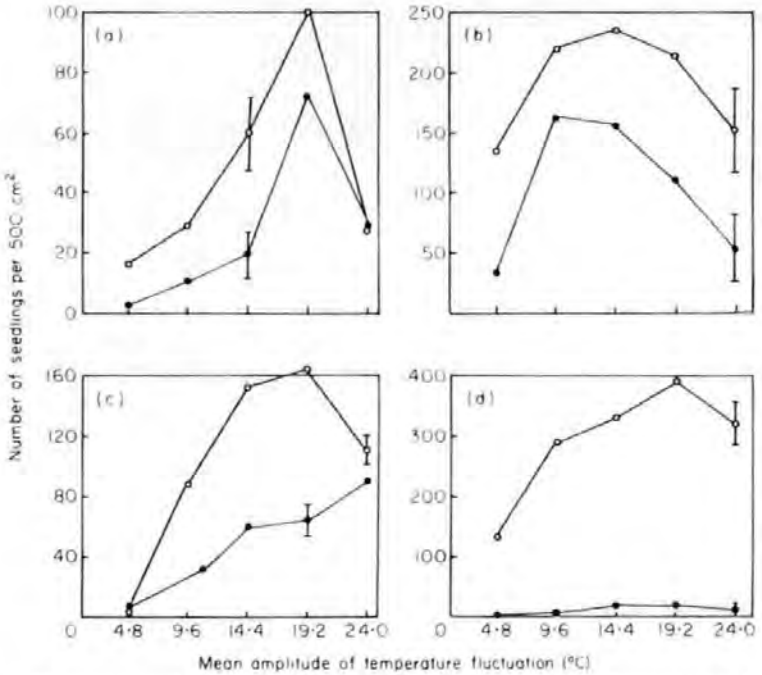

Fig. 4. The response of naturally-buried seeds to diurnal temperature fluctuations in light (○) and darkness (●). The numbers of seeds germinating in the light have been adjusted to take account of the higher seed concentrations in the dark due to extra sieving. (a) *Poa annua*, (b) *Stellaria media*, (c) *Polygonum aviculare*, (d) *Juncus acutiflorus*. Vertical bars represent the least significant difference between treatment means ($P = 0{\cdot}05$).

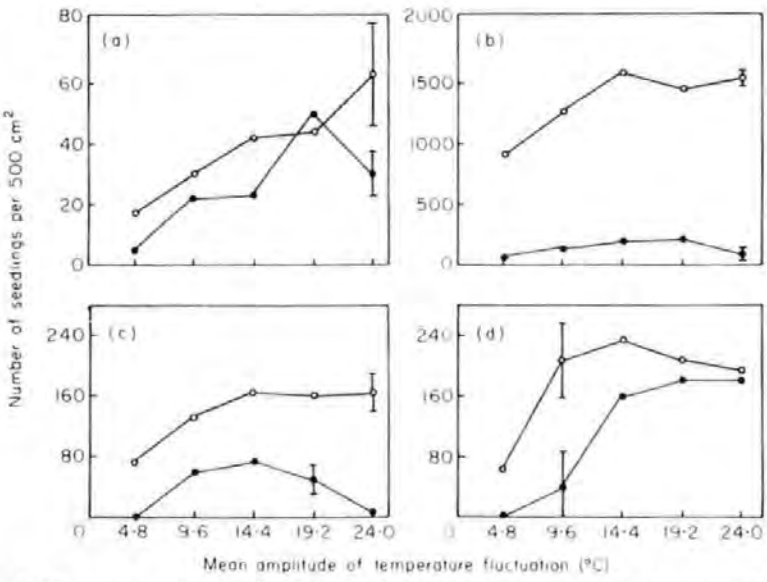

Fig. 5. The response of naturally-buried seeds to diurnal temperature fluctuations in light (○) and darkness (●). The numbers of seeds germinating in the light have been adjusted to take account of the higher seed concentrations in the dark due to extra sieving. (a) *Agrostis stolonifera*, (b) *Epilobium tetragonum*, (c) *Cardamine hirsuta*, (d) *Coronopus didymus*. Vertical bars represent the least significant difference between treatment means ($P = 0{\cdot}05$).

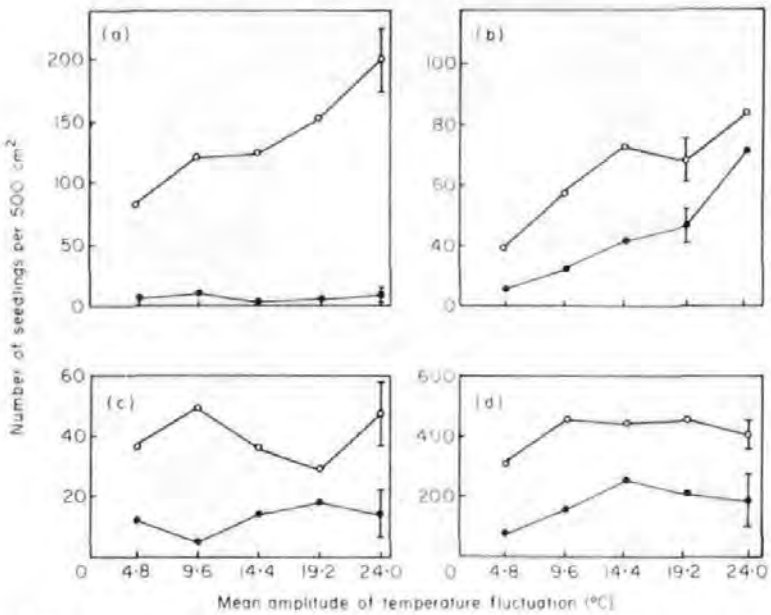

Fig. 6. The response of naturally-buried seeds to diurnal temperature fluctuations in light (○) and darkness (●). The numbers of seeds germinating in the light have been adjusted to take account of the higher seed concentrations in the dark due to extra sieving. (a) *Hypericum perforatum*, (b) *Plantago major*, (c) *Spergula arvensis*, (d) *Digitalis purpurea*. Vertical bars represent the least significant difference between treatment means ($P = 0{\cdot}05$)

behaviour found within a single population of seeds, which has already been demonstrated for harvested seeds (Thompson & Grime, 1983), was again evident. In most species, this took the form of a smooth increase in the number of seeds germinating as the amplitude of temperature fluctuation increased. In a minority of species there was a more obvious 'step' in germination between mean amplitudes of 4·8 and 9 °C [e.g. *R. obtusifolius*, Fig. 7(a) and (c), and *Coronopus didymus*, Fig. 5(d)]. There was a suggestion in some species [e.g. *A. stolonifera*, *Hypericum perforatum*, Fig. 6(a), and *Plantago major*, Fig. 6(b)] that a further increase in the amplitude of fluctuation above 24 °C, the maximum employed here, could have resulted in a further increase in germination.

Responses to temperature fluctuations in the dark

It can be seen from the figures that, with few exceptions, the effect of darkness was to reduce the number of seedlings emerging from a soil sample, even at large temperature fluctuations. Some species [e.g. *Hypericum perforatum* and *Juncus acutiflorus*, Fig. 4(d)] showed almost no germination in the dark, irrespective of temperature regime. In several species (e.g. *E. tetragonum*, *Poa annua*, *Plantago major*) the response to increasing amplitudes of temperature fluctuations was more gradual in the dark than in the light. The shape of the response curves to increasing temperature fluctuations was often different in the dark from that in the light, the main difference being a narrower optimum temperature range for germination in the dark. The drop in seedling numbers emerging when the amplitude of temperature fluctuations exceeded 19 °C was generally more marked in the dark than in the light [e.g. *Cardamine hirsuta*, Fig. 5(c), *A. stolonifera* and *Holcus lanatus*,

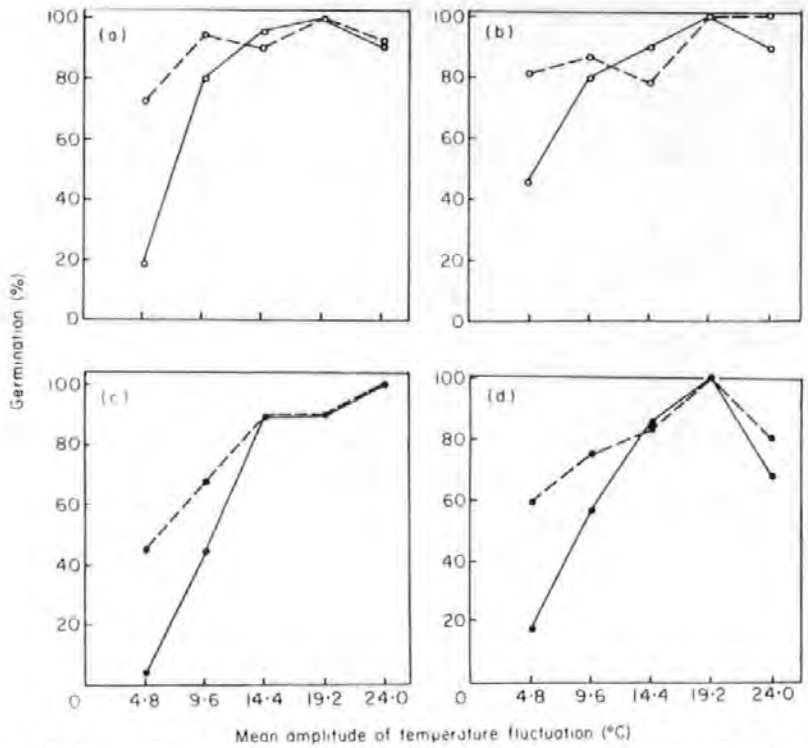

Fig. 7. The response of naturally-buried seeds and of harvested dry stored seeds to diurnal temperature fluctuations in light (○) and darkness (●). In order to compare buried and harvested seeds all figures are expressed as percentages of maximum germination obtained in any particular experiment. (a) and (c) *Rumex obtusifolius*, (b) and (d) *Holus lanatus*. ——, Buried seeds; – – –, harvested seeds.

Fig. 7(b) and (d)], but *Polygonum aviculare* and *R. obtusifolius* were exceptional in this respect.

Response of harvested seeds to temperature fluctuations

The results described above can be compared with those in Thompson & Grime (1983) for harvested seeds of the same species. However, since the temperature regime used in their experiments was not the same as that described here exact comparison is not possible, for differences in germination responses between the two sets of data could be due to the different temperature regimes rather than differences in the response of harvested and naturally-buried seeds. For the two species shown in Figure 7, however, it was possible to make a more exact comparison between the behaviour of harvested and naturally-buried seeds.

The harvested seeds showed considerably more germination at low amplitudes of temperature fluctuations than buried seeds [e.g. buried *R. obtusifolius* showed 20% germination at 4·8 °C fluctuation but harvested seeds showed 70% germination, Fig. 7(a)]. This was found both in the light and in darkness. At amplitudes of temperature fluctuations greater than 10 °C the germination responses of harvested and buried seeds were very similar.

Discussion

Effectiveness of the methods

The study of the germination requirements of naturally-buried seeds presents many practical difficulties, only some of which have been wholly overcome by using the technique described. First, the act of collection itself may have altered the seeds' dormancy states by its effect on soil atmosphere and by abrading some seeds. However, Karssen (1981a) has shown that oxygen and carbon dioxide levels in soil are unlikely to be significantly different from those in air. Similarly Holm (1972) suggests that volatile organic compounds are only likely to be important in compacted soil or at depths greater than those from which soil was collected for this study. Wesson & Wareing (1969) have shown that abrasion of seeds during soil collection does not affect subsequent germination.

Drying of soil after collection was necessary in order to allow manipulation of the soil without exposing imbibed seeds to light. There is clearly a possibility that this drying may alter the dormancy state of seeds in soil. However, the surface layers of soil dry out to a considerable degree during dry spells even in Britain, and it seems likely that the artificial drying carried out here does not depart from the normal experience of many buried seeds.

Attempts to maintain the entire dark thermogradient bar in complete darkness for 14 d produced etiolated seedlings which were susceptible to 'damping-off' disease and were difficult to identify. A layer of sand covering the soil, together with exposure to dim white light only, kept the seeds in darkness but enabled the seedlings to grow through into the light. Controlled experiments, in growth cabinets, demonstrated no significant difference between seedling emergence from bare soil in total darkness and soil covered with sand in dim light.

The depth of soil used on the bars was a compromise between (a) a layer thick enough to prevent rapid drying and containing sufficient seeds, and (b) a layer thin enough to be uniformly affected throughout its depth by the temperature fluctuations and for most of it to receive light when unprotected by sand. A 5 mm layer came closest to satisfying all these requirements. The ped size produced by the soil preparation was quite large and this aided penetration of light (Woolley & Stoller, 1978).

As in the case of soil depth, the temperature regime employed on the bars (Fig. 2) represents a compromise between conflicting requirements. Seeds in different positions on the bars experience differences in (a) temperature fluctuation, (b) extremes of temperature and (c) mean temperature. Points (a) and (b) are discussed fully below, but (c) can be dealt with more briefly here. Ideally one would like to eliminate variation in mean temperature along the bars, either by raising the 'constant' temperature or by lowering the lower temperature experienced at large fluctuations (refer to Fig. 2). In fact raising the constant temperature would result in the smaller fluctuations occurring at temperatures far higher than are ever associated with such fluctuations in the field. Lowering the lower extreme temperature would reduce it to 0 °C or below, which is equally undesirable. Using the regime adopted (Fig. 2) it seems possible that the mean temperature of the section nearest the constant end (13·6 °C) is just below the *constant* temperature at which a small minority of the species tested could be expected to germinate to a high percentage (Grime *et al.*, 1981). However, it must be remembered first, that a significant part of each day was spent at a temperature above 13·6 °C. Secondly, and more importantly, even this section experienced a mean fluctuation of 4·8 °C,

and it is well known that the lower temperature limit for germination can be extended downwards quite a long way by relatively small temperature fluctuations (Grime *et al.*, 1981; Thompson & Grime, 1983). We suggest, therefore, that the variation in mean temperature along the bar probably has no practical effect on germination.

Interaction between amplitude of fluctuation and absolute temperature

It is often difficult to distinguish between responses to fluctuations *per se* and to the absolute temperature extremes experienced during those fluctuations. Some progress can be made by comparing the results obtained here with the constant temperature limits for 50% of maximum germination in the light, as established for harvested seeds by Grime *et al.* (1981). In *Poa annua* there is a decline in germination at large fluctuations, yet the upper and lower limits for germination at constant temperatures are very wide, suggesting the reduction is an effect of fluctuations *per se*. In *S. media* inhibition of germination at large fluctuations does correspond to exposure to temperatures outside the limits for high germination at constant temperatures. In contrast, in *Cardamine hirsuta* and *R. obtusifolius*, absolute temperatures, both above and below those conducive to high germination at constant temperatures, are not associated with a reduction in germination when these temperatures form part of a fluctuating regime.

Constant temperature limits for germination in the dark are not known, but Totterdell & Roberts (1980) have shown that *R. obtusifolius* enters induced dormancy very rapidly above 25 °C, especially in the dark. There is no evidence of this in the present study, even when the fluctuating regime includes temperatures well above 25 °C. The only safe conclusion to be drawn at present seems to be that fluctuating temperatures have effects difficult to predict from the effects of their component absolute temperatures.

Interaction of light and fluctuating temperatures

At any given amplitude of temperature fluctuation more seeds germinated in the light than in the dark, but stimulation by fluctuating temperatures was still evident in the light, i.e. light could not entirely substitute for fluctuating temperatures. The very different shapes of light and dark germination curves in several species suggest that this is a real effect and not an artefact arising from limited penetration of light into the soil in the light treatment. This is in contrast to the results of Thompson & Grime (1983), using harvested seeds, in which a requirement for fluctuating temperatures in several species (e.g. *Poa annua* and *Holcus lanatus*) was abolished by light. It therefore seems that burial may induce not only a requirement for light (Wesson & Wareing, 1968) but also a requirement for fluctuating temperatures.

Differences between harvested and buried seeds

When the germination responses of naturally-buried and harvested seeds of the same species were compared, a smaller proportion of buried seeds germinated at small amplitudes of temperature fluctuations (Fig. 7). Dry storage of harvested seeds may cause a reduction in dormancy (Bewley & Black, 1982), but this is unlikely to have been important here since the harvested seeds were stored for a relatively short period of time at a low temperature which retards loss of dormancy (Cavers, 1974; Totterdell & Roberts, 1979). This lack of response to low temperature fluctuations in buried seeds seems therefore to be a real difference

from the situation in freshly shed seeds, and may have arisen in either of two ways. First, as suggested above, exposure to conditions during or perhaps immediately before burial may have induced or increased a requirement for fluctuating temperatures. Secondly, the seed population may contain a minority of seeds which require large fluctuations when freshly shed. Rapid germination of the majority lacking this requirement may have left the persistent buried seed population enriched in seeds requiring fluctuations. Support for the first alternative comes from recent work (Whatley, unpublished) which suggests that a requirement for fluctuating temperatures can be artificially induced.

Ecological implications

Much of what has already been written on this topic, in the context of germination tests on harvested seeds (Thompson & Grime, 1983), is also relevant here. It is clear that partial or total inhibition of germination by darkness is ubiquitous among species with persistent seed banks, and that this inhibition is most marked in species with very small seeds (e.g. *J. acutiflorus*, *E. tetragonum*, *Hypericum perforatum*). Nevertheless this relationship, which is presumably correlated with the limited ability of small seedlings to penetrate more than a shallow layer of soil, is not always found. Germination of the very small seeds of *A. stolonifera* is scarcely reduced by darkness, perhaps (although this is largely speculative) because grass seedlings are better able to penetrate soil than dicotyledonous seedlings.

Stimulation of germination by fluctuating temperatures is also very common, though not universal, in buried seeds. The adaptive value of this as a depth-sensing and gap-detecting mechanism has been discussed previously (Thompson & Grime, 1983). One effect of burial, already mentioned above, seems to be to induce a requirement for fluctuating temperatures or to increase an existing requirement. Whatever the origin of a fluctuating temperature requirement, however, one of its major effects will be to restrict the germination of seeds beneath a closed canopy to gaps in that canopy. In one experiment in ryegrass pasture, for example, few naturally-buried *Holcus lanatus* seeds germinated in 10 and 15 cm diameter canopy gaps, but many more germinated in 25 cm gaps (Thompson, 1977, cited in Grime, 1979, p. 96).

A rather unexpected feature of the results is the inhibition of germination by large fluctuations in several species. Admittedly not all aspects of an organism's biology are necessarily adaptive and inhibition of germination by large fluctuations may be an inevitable consequence of other adaptive features of the plant's physiology. Nevertheless extreme fluctuations are probably always associated with hot weather and drying soil and inhibition by these fluctuations may have the selective value of preventing germination under such conditions.

Further applications of the thermogradient apparatus

There is abundant evidence that some species undergo seasonal cycles of dormancy, and clearly the apparatus is well suited to the study of seed populations at regular intervals over a period of months or years. The apparatus is also suitable for use with soil or other growth media into which known numbers of seeds have been introduced, allowing more precise statements about the germination percentages associated with particular treatments. The potential for comparison of harvested and naturally-buried seeds is much greater than has actually been realized in the study reported here. In particular it should be possible to apply

particular pre-treatments to harvested seeds in an attempt to duplicate the behaviour of buried seeds. Finally, it may prove possible to increase the 'realism' of the experimental seed environment by pumping air of varying composition through the chambers and thus simulate the effect of different depths of burial on soil atmosphere.

ACKNOWLEDGEMENTS

We would like to thank Dr E. A. Vallis and Dr G. D. Wigham for their advice on the design of the apparatus, and Mr L. F. Cook and Mr A. Hopkins for their technical assistance. Financial support was provided by Devon County Council.

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